An experimental and descriptive analysis of the responses and peripheral and central organisation of the locust metathoracic femoral chordotonal organ - a leg joint position and movement detector

Thomas Matheson

Department of Zoology
University of Canterbury
Christchurch
New Zealand

A thesis submitted in partial
fulfilment of the requirements
for the degree of
Doctor of Philosophy in Zoology
in the
University of Canterbury

October 1990.

ABSTRACT

Extracellular dye infusions, light microscopy, electron microscopy, and intracellular recording and staining have been used to physiologically and morphologically characterise individual sensory neurones of a locust joint stretch receptor, the metathoracic femoral chordotonal organ (mtFCO).

A new population of receptor neurones is described, approximately doubling the number of neurones known in the mtFCO. These distally located neurones appear to represent a group previously thought to be missing in the hind leg.

The positions of individually characterised neurones' somata within the chordotonal organ are mapped in detail, and are shown to be related to aspects of each neurone's response to tibial movements. Neurones respond to position, velocity, or acceleration, or to combinations of these parameters. Both extension and relaxation sensitive neurones are found throughout the organ. Movement of the main distal attachment of the organ (the apodeme ligament) stimulates both types of receptors. Range fractionation of tonic and phasic responses, and hysteresis of tonic firing are described and illustrated in detail, and interpreted in terms of function.

The central projections of mtFCO neurones are shown to differ between response types. Neurones within at least some response types also show gradations in their patterns of branching which can be related to the leg angle which causes their maximal tonic or phasic firing. This is similar to, but not the same as tonotopic mapping described previously for auditory chordotonal organs.

A novel use of discriminant and cluster analyses to assist the description of neuronal morphology is presented.

CONTENTS

ERRATA

The following is a list of errors discovered after submission and examination of the thesis.

Page	Para.	Line	Correction
1	2	4	flexor-tibia = femur-tibia
9	2	2,9	Field submitted = Field in press
9	3	2	3 FCO = FCO
		7,9	submitted = in press
10	2	12	*insert*: Bräunig termed this posterior ventral strand *vlig*
		21	submitted = in press
18	3	14	My third = My fourth
56	3	2	$P^{(20}V^{+)}?A^{-}$ = $P^{(20)}V^{+}?A^{-}$
	6	1	$P^{(20)}V^{+}?A^{-}$ = $P^{20}V^{+}?A^{-}$
58			*Add to figure caption*: (Figure 19B overpage)
66	2	4	Zill 1985 = Zill 1985a,b
69	Caption		(D) *Circles* = $P^{0}V^{+}?A^{+}$
75	Caption		P120V-?A- = $P^{120}V^{-}?A^{-}$
75	Caption		P120V- = $P^{120}V^{-}$
76	1	6	animal = neurone
	2	1	phasic-tonic = phasic
	2	6	4 spikes 200 Hz = 4 spikes at 200 Hz
79	Caption		°s-1 = $°s^{-1}$
79	3	6	20°s-1 = $20°s^{-1}$
83	1	10	Fig. 17 and inset = Fig. 17 inset
90	3	4	Fig. 9D = Fig. 4D
92	2	1	*delete:* Table 1
		4	6D = 1D
		8	7A = 2A
		13	8D = 3D
94	2	3	(Compare Fig. 16A & B) = (Fig. 15)
99	2		point 5: ventral branches were not used in the final analysis
105	1	6	3Biv = 3Biii
112	Caption		Y = -0.063 + 12.1 *becomes* Y = -0.063X + 12.1
113	5	11	V+ = V^{+}
114	Caption		Fig. 13A,B overpage = Fig. 14A,B overpage

The following references were omitted from the reference list:

Bate CM (1978) Development of sensory systems in arthropods. In Jacobson M (ed) *Handbook of Sensory Physiology.* Springer-Verlag New York. pp. 2-53.

Field LH (in press) Mechanism for range fractionation in an insect chordotonal organ. *Internat. J. Insect Morph. Embryol.*

Forman RR, Zill SN (1984) Leg position learning by an insect: II. Motor strategies underlying learned leg extension. *J. Neurobiol.* 15: 221-237.

Mann DW, Chapman KM (1975) Component mechanisms of sensitivity and adaptation in an insect mechanoreceptor. *Brain Res.* 97: 331-336.

Taylor RC (1975) Integration in the crayfish antennal neuropile: Topographic representation and multiple channel coding of the mechanoreceptive submodalities. *J. Neurobiol.* 6: 475-499.

GENERAL INTRODUCTION

The study of neural function encompasses a wide range of topics. Investigations of membrane channel kinetics and the genetics of nerve growth help form the 'molecular' end of this spectrum, while the other end comprises the large body of work which considers neuroethological questions. In response to the varied needs of different researchers, a range of well-studied animal 'preparations' have been developed. Specific preparations are usually designed (at least initially) to allow investigation of particular problems. 'Successful' preparations develop over time as a body of knowledge is built up around these initial findings. Characteristically, researchers new to a field find it convenient to begin their research by building on the knowledge derived from an established (and therefore successful) preparation. This eliminates the need for extensive and time-consuming 'background' research. The outcome of this tendency is that our understanding of specific neural processes or phenomena is based on discrete areas of research, often on very dissimilar animals. At points along the way there must be attempts to synthesize related research so that meaningful comparisons can be made, and similarities and differences highlighted. Such syntheses should provide many new ideas for future research. The locust preparation used as the basis for my study belongs to this 'successful' category. It was popularised by Hoyle (e.g., 1964), and has been used by many researchers in a wide range of studies since then. Locusts are good subjects because they can exhibit complex behaviour; they are easily obtainable; and when dissected, they can be maintained for long periods of time in a condition suitable for neurophysiological recording.

SPECIALISATION OF LOCUST LEGS

Locust's metathoracic (hind) legs are considerably larger than their pro- and mesothoracic counterparts (Fig. 1), in terms of both length and weight. Because the hind legs move in a plane almost parallel to the body during walking, extension of the metathoracic flexor-tibia joint is functionally equivalent to retraction of a pro- or mesothoracic leg: i.e., this is the power or stance phase (Burns 1973). In contrast to the forelegs, where propulsive forces are largely generated at the body-coxa joint, most of the propulsive force in the hindleg is generated by the tibial extensor muscle (i.e., at the femur-tibia joint) (Burns & Usherwood 1979). Not only are the metathoracic tibial flexor and extensor muscles larger than their counterparts in the forelegs, but the relative sizes are reversed: in the hindleg the extensor is larger than the flexor. This probably reflects their changed roles in locomotion (Burns & Usherwood 1979). Differences

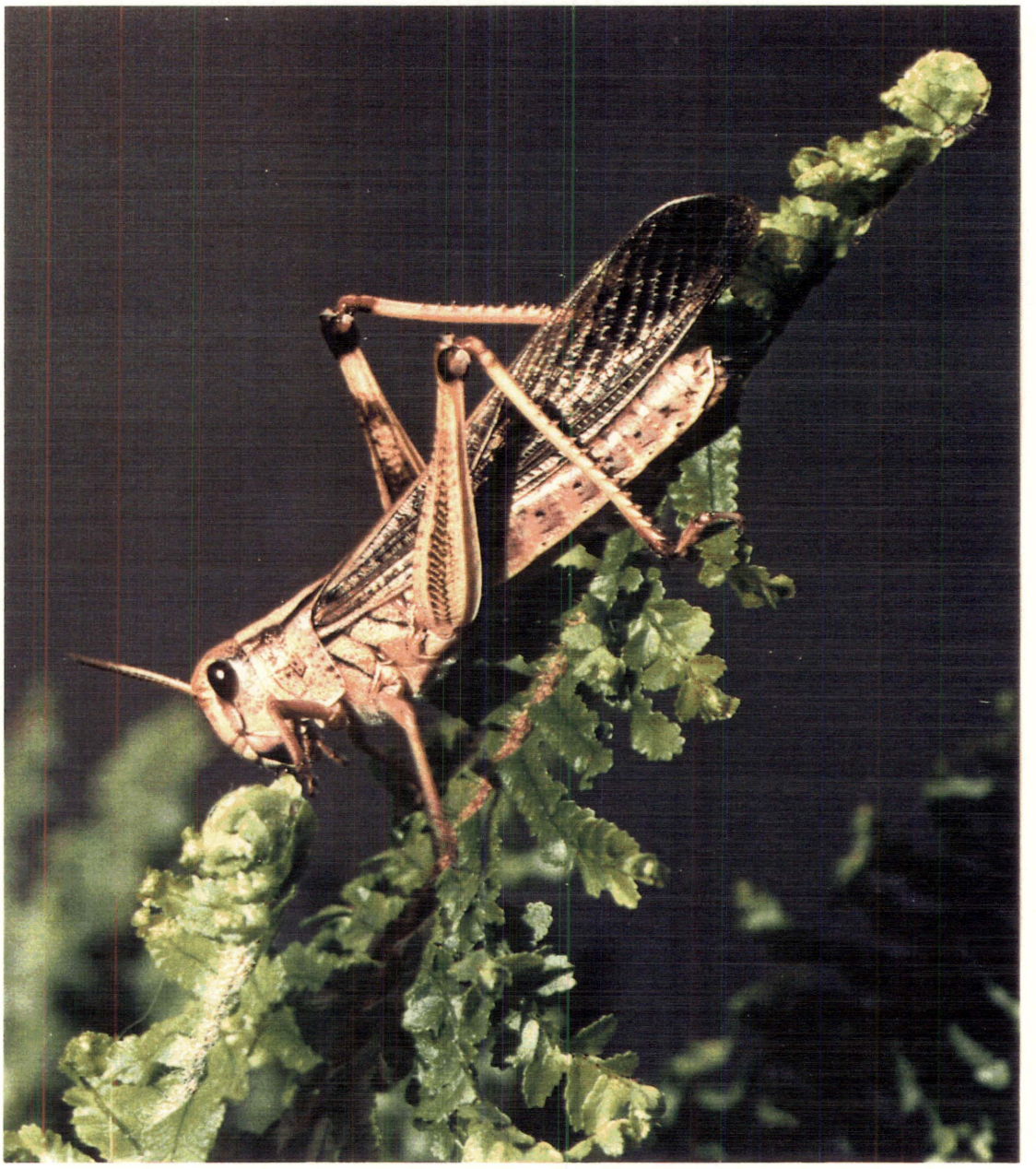


Figure 1. Adult female locust (*Locusta migratoria*). The metathoracic (hind) legs are much larger than the forelegs, and are moved in a plane parallel to the body. This animal is approximately 6 cm long.

between the metathoracic leg and the other legs regarding innervation (ratio of fast to slow motorneurone endings), muscle fibre distribution (phasic versus tonic fibres), and axon paths (leg nerves followed by fast and slow extensor motorneurones) probably result from the adaption of the locust for jumping (Burns & Usherwood 1979).

During normal walking there is considerable variation in the movement and coordination of all the legs, with the pro- and mesothoracic legs being respectively the most, and least variable. Burns (1973) attributes much of this variability to the 'disruptive' influence of the metathoracic leg, which can easily be induced to flex in preparation for a jump even while the animal is walking.

Movements and stresses at insect femur-tibia joints are monitored by a number of sensory structures. These include campaniform sensilla (Zill & Moran 1981), multipolar joint receptors (Bässler 1977, Coillot & Boistel 1968, 1969), tension receptors (Bässler 1977) and probably most importantly, the femoral chordotonal organ (FCO) (Bässler 1977, Burns 1974, Burrows 1988, Büschges 1989, Field & Burrows 1982, Field & Rind 1981, Hofmann & Koch 1985, Hofmann et al. 1985, Theophilidis 1986a,b, Usherwood et al. 1968, Zill 1985a,b).

THE IMPORTANCE OF PROPRIOCEPTIVE FEEDBACK

Animal behaviour must be flexible in order to cope with variations in the environment. External sensory structures of many kinds permit animals to perceive their surroundings (e.g., mechanosensors, thermosensors, chemosensors), while internal sense organs such as joint chordotonal organs monitor the animal's own movements (proprioceptors).

The most important role of proprioceptors is to allow flexibility of behaviour. Neuronal systems can (and do) produce stereotyped motor outputs in some situations, but the majority of behaviours, especially complex sets of movements must be shaped by proprioceptive feedback. In order to explain behaviour in terms of neural activity we must understand proprioceptive systems at several levels: (1) what information is monitored; (2) how is the information obtained (what is the mechanism of sensory transduction, and how do the mechanical or electrical properties of neurones and supporting structures filter the original stimulus); (3) where does the information go (what motorneurones and interneurones do the proprioceptors make functional connections with); and (4) what are the integrative and output properties of these other neurones. Such an understanding is clearly important, and can only come from detailed studies of

each component process. My research investigates some of these questions in a complex multineuronal sense organ, the locust metathoracic femoral chordotonal organ (mtFCO).

CHORDOTONAL ORGAN OVERVIEW

Chordotonal organs are internal sensory structures which respond to movement or degree of stretch. They are present only in the insects and crustaceans (Moulins 1976). Their sensory cells are associated with complex ciliated mechanotransducer structures called scolopidia (see later). Connective chordotonal organs (Howse 1968) consist of one, or up to several hundred such bipolar neurone somata embedded in a sheet (or sheets) of connective tissue which span a joint, or connect two structures (for reviews see Mill 1976). Chordotonal organs are known from all the leg joints, and from the antennae (including Johnston's organ), the wing bases, and the haltares. Subgenual organs (vibration receptors in the legs) and tympanal (auditory) organs of the legs, abdomen, or thorax are also specialised chordotonal organs. See Bullock & Horridge (1965) and authors in Mill (1976) for reviews and references.

Scolopidia are characteristic structures of chordotonal organs, and have been the focus of a great deal of interest (see Moulins (1976) for review). Their detailed structure has been examined by many authors, including Ball (1981), Füller & Ernst (1973), Mill & Lowe (1973), Slifer & Sekhon (1975), Young (1970), and most recently, Wolfrum (1990), and is illustrated in Fig. 2. Depending on the species and the chordotonal organ, a single scolopidium may contain the dendrites of either one or more sensory neurones (compare left and right in Fig. 2). In addition to these sensory cells, each scolopidium consists of a distal *attachment cell* (or cap cell), a *scolopale cell*, and possibly other supporting cells (e.g., *sheath cell*) (Fig. 2).

The highly vacuolated scolopale cell surrounds the distal portion of the dendrites. During development it secretes a series of *scolopale rods* which form a barrel-like structure surrounding the dendrites. Distally these rods insert into another extracellular structure (also secreted by the scolopale cell): the end cap or *scolopale cap*. This is in turn surrounded by the microtubule-filled attachment cell. This cell provides a distal mechanical link to the supporting cuticle.

The sensory dendrites contain a *cilium* which inserts into the scolopale cap inside the ring of scolopale rods. The cilium has a distinctive *ciliary dilation* near its distal end, and *basal bodies* proximally where it arises from the dendrite.

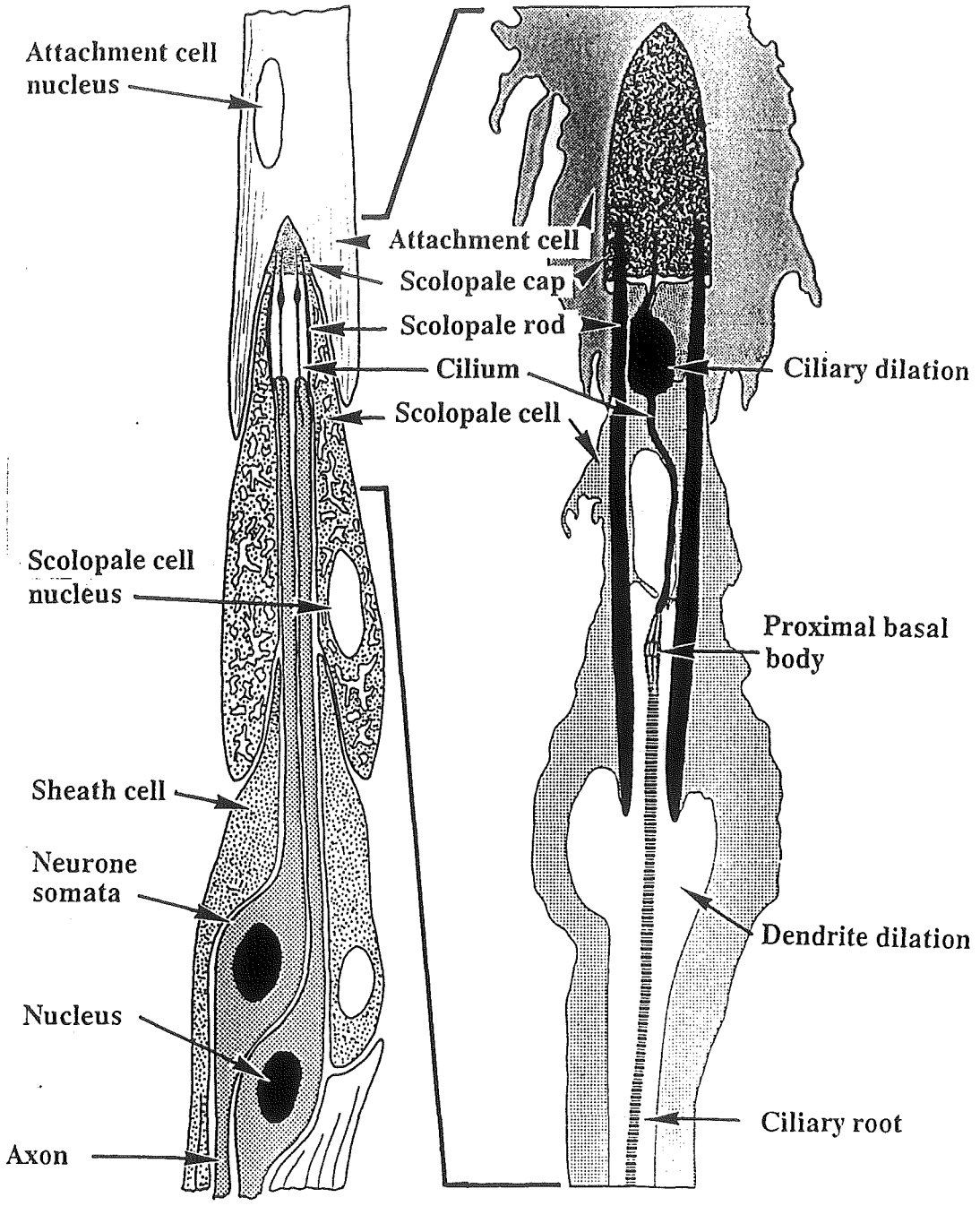


Figure 2. Generalised ultrastructure of chordotonal organ scolopidia. The drawing to the right is an enlargement of the area indicated by the solid lines, but is from a scolopidium with only a single dendrite. Both figures are schematic, and therefore not to scale. Structures are described in the text. Redrawn from Füller & Ernst (1973), and Ball (1981) respectively.

Scolopidia are the primary site of mechanical to electrical transduction in chordotonal organs, but, as yet, no consensus has been reached about their adequate stimulus, or exact mode of action. A variety of possibilities have been proposed: Howse (1968) suggested stretch of the ciliary dilation by flexion at the

scolopale/cap junction; while Moran et al. (1975, 1977) proposed that bending of the ciliary dilation caused an 'active stroke' or 'active sliding' in the cilium which in turn distorts the cell membrane near the basal body. Young (1970) states that the adequate stimulus in the cockroach tibio-tarsal organ appears to be stretch of the ciliary apparatus. Zill (1985a) proposed that in the locust mtFCO stretching and bending is the adequate stimulus for tonic receptors, while phasic receptors must respond to more complex stimuli, possibly including relaxation.

Moulins (1976) and Mill (1976) state that differences in the composition of attachment strands explain relaxation or extension sensitivity (i.e., relaxation sensitive neurones have scolopidia embedded in strands rich in connective tissue, while elongation sensitive neurones have scolopidia embedded in cellular strands which are poor in extracellular connective tissue). Recently however, Field (submitted) has pointed out that although both response types are known in the locust mtFCO (Matheson 1990, Zill 1985a) and in the locust coxal CO (Hustert 1982), their strands are of the cellular type. Details of the functioning of CO scolopidia therefore remain unclear.

LOCUST FEMORAL CHORDOTONAL ORGAN STRUCTURE

Prothoracic and mesothoracic legs

In the locust, the prothoracic and mesothoracic FCOs (prFCO and msFCO respectively) are located proximally in the femur, and are connected to the tibia by a long apodeme (Burns 1974). In addition, each is divided into two distinct groups of neurone somata: the proximal and distal scoloparia. Some authors have described these two groups as separate chordotonal organs (Moran et al. 1975), but this is not the usual usage, since both the scoloparia join onto the common apodeme. In different insect species, or even in different legs, there are differing degrees of fusion between the two scoloparia (Field & Pflüger 1989), ranging from almost complete fusion in the New Zealand weta (Orthoptera), to complete separation in the locust. The stick insect (Bässler 1983) and bush cricket (Theophilidis 1986b) both have partially fused scoloparia.

In insects which have been investigated (e.g., locust, stick insect, bush cricket), the two scoloparia are morphologically and physiologically different. The locust proximal scoloparium contains "several hundred" small, uniform sized cells (3-11 μm), while the 42 neurones of the distal scoloparium range in

size between 15 and 40 μm (Field & Pflüger 1989). In the locust, but not in most other orthopterans, the cells of the proximal scoloparium are arranged in a regular conical array which resembles the structure seen in chordotonal organs of the cicada vibratory tymbal (Young 1975), or those of the subgenual or Johnston's organs (Burns 1974). Neurones of the corresponding scoloparium in the tettigoniid *Decticus*, though, show the usual pattern: they are scattered throughout the connective tissue band (Theophilidis 1986a). Figure 3 (adapted from Field & Pflüger 1989) illustrates the location of the locust msFCO in the femur, and the arrangement of the neurone somata in the two scoloparia.

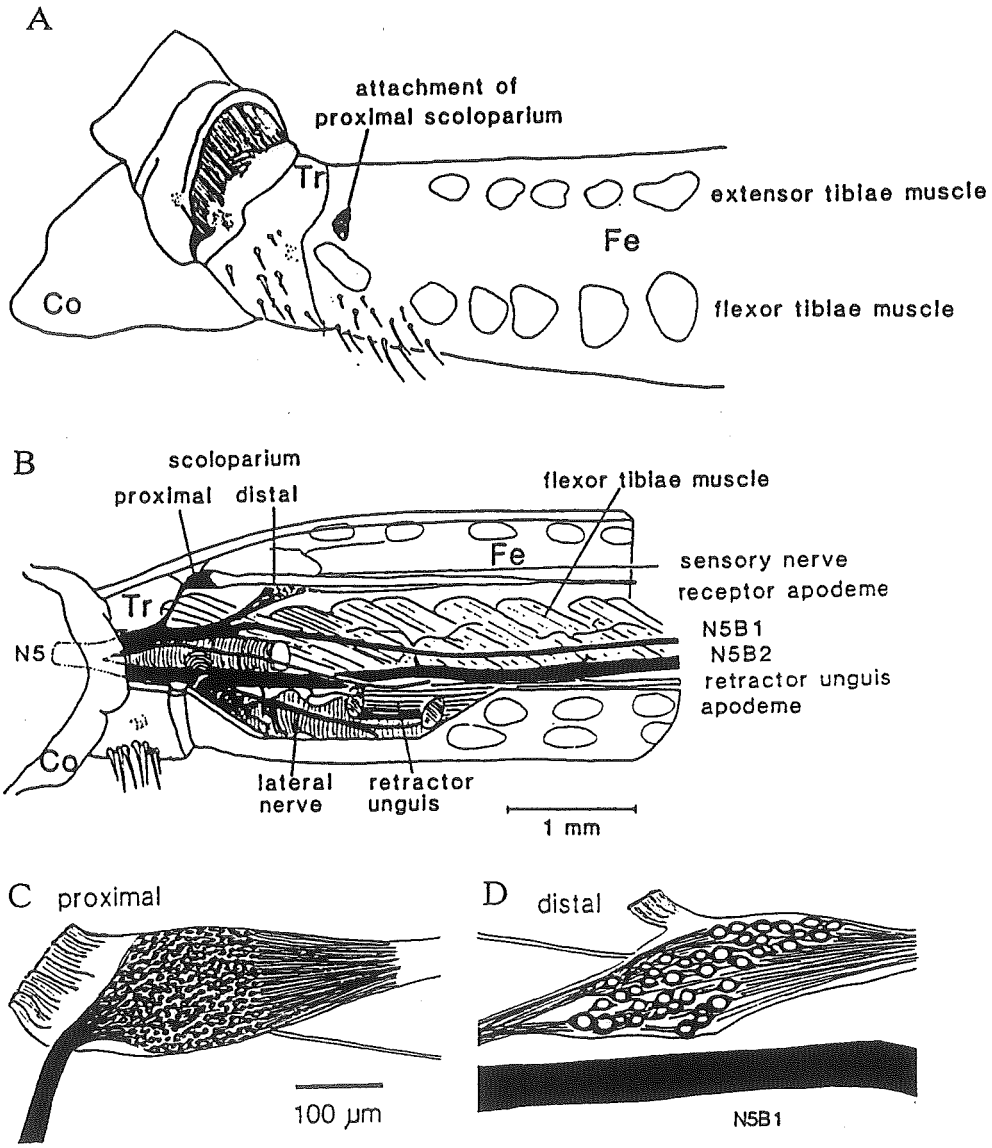


Figure 3. Structure of the mesothoracic femoral chordotonal organ. (A) The site of attachment is visible in this anterior view of the proximal leg joints as a dark patch on the cuticle. (B) Peripheral cobalt backfill of both scoloparia and leg nerves. (C,D) enlargements of the proximal and distal scoloparia respectively. All drawings taken from Field & Pflüger (1989).

Metathoracic leg

The hind leg femoral chordotonal organ of the locust is superficially quite different to those of the other legs, and to the same organ in *Decticus* (Theophilidis 1986a,b). It is located distally in the leg, and is connected to the tibia by a rather short (3 mm) ligament (Fig. 4). The main proximal attachment is by a short stout ligament to the anterior dorsal cuticle. The mtFCO is composed of a single scoloparium, consisting of between 24 and 55 neurones (counts by Usherwood et al. 1968 and Zill 1985a respectively). Axons from these neurones coalesce into a short nerve (termed the chordotonal nerve by Usherwood et al. (1968)), which joins n5B1 (Campbell 1961) within 500 *u*m of the chordotonal organ (Burrows 1987). This nerve also contains axons which run past the mtFCO to innervate hairs on the distal femur (Zill 1985a). The cell bodies within the organ are not arranged in a regular array, but often appear to be grouped into 2 (Zill 1985a) or 3 (Usherwood et al. 1968) regions when viewed in whole-mount under the light microscope. Zill (1985a) stated that the scolopidia are similarly grouped: being either parallel to the axis of the leg (neurones with dorsal somata) or at an angle of 30-35° (ventral somata).

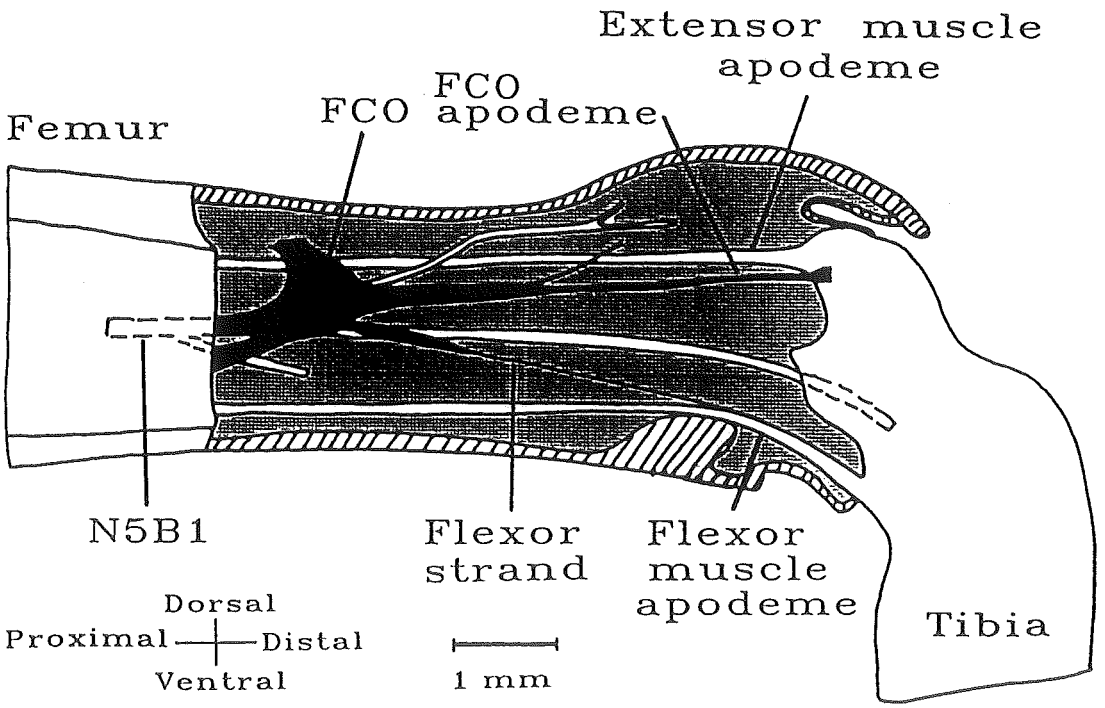


Figure 4. View ot the distal metathoracic femur illustrating the location of the mtFCO. Structures are described in the text. Modified from Field (in press).

Distally the mtFCO is connected to the tibia by two main attachments: a main ligament which inserts onto a dorsal cuticular apodeme (which meets the tibia near the extensor muscle apodeme), and a somewhat finer structure termed the flexor strand which inserts ventrally onto the apodeme of the flexor muscle. The main ligament is a multicellular strand composed of bundles of microtubule-filled attachment cells. Each attachment cell connects a single scolopidium in the body of the organ to the distal cuticle (apodeme). It is useful to consider the mtFCO as 3 units in series between the cuticle of femur and the tibia: the FCO proper; the ligament; and the apodeme.

The ligament is divided into 2 main bundles close to the apodeme: the e (end) and s (side) bundles (Field submitted). The e bundle attaches directly to the end of the apodeme, where it appears to contain a specialised 'hinge'. When the tibia is fully flexed this bundle is taut, but it quickly begins to relax and buckle as the tibia extends. In contrast, the s bundle subdivides before attaching in a graded series of small bundles along the proximal 900 *u*m of the apodeme. These small bundles remain taut over a wider range of leg angles, and are under differential tension. In other words, as the tibia extends, tension is released from these strands in a graded manner so that some strands remain taut while others become slack (strand recruitment). Field (submitted) proposes that this complex attachment, and the consequent strand recruitment is a mechanical basis for at least some aspects of range fractionation in the mtFCO. All of the neurones in the mtFCO have scolopidia and attachment cells in the main ligament.

The second main distal attachment of the mtFCO is the flexor strand (FS). This fine elastic structure leaves the body of the 3 FCO ventral to the main ligament, and runs distally to connect to the apodeme of the flexor tibiae muscle approx 5 mm proximal to the tibia. Because of its great elasticity, the flexor strand is under at least some tension at all leg angles. The ultrastructure of the flexor strand has not been investigated in detail, but it is clear that it differs from the main ligament (Field submitted). There are no scolopidia associated directly with the FS, so it cannot be composed of attachment cells. Field (submitted) has shown that it fans out over the outer neural lamella of the FCO, and does not appear to have any direct connections to the neurones or scolopidia. The FS contains a single multipolar stretch receptor (Bräunig 1985), which responds to extension of the tibia (stretch of the flexor strand). This receptor is active at all leg angles except full flexion (0°). The strand receptor is known to synapse with a spiking local interneurone of the midline group (Pflüger & Burrows 1987), but

its reflex effects (if any) have not been described. Stimulation of the flexor strand caused reflex effects in some leg motorneurones (Field & Burrows 1982), but it is not clear whether they were caused by the FCO or the strand receptor (Field pers. comm.).

There is some confusion about other possible attachments of the mtFCO to the tibia, and to femoral muscles. Usherwood et al. (1968) illustrated 2 additional connections: one a band-like structure which connects to the flexor muscle apodeme proximal to the FCO; the other a very fine strand which branches from the main ligament close to its attachment to the apodeme, and connects distally to fibres of the extensor muscle. Zill (1985a) states specifically that these attachments could not be found in his sectioned preparations, and suggests that the fine strand described by Usherwood et al. (1968) is a nerve. Recent work by Field (in press), and my present study have confirmed the existence of both structures described by Usherwood et al. (1968). The large posteriorly directed attachment to the flexor muscle apodeme has not been examined in detail, but is certainly exists. The fine strand which leaves the main ligament to the apodeme was noted in passing during an SEM study conducted as part of my research, and has been noted previously by Field (pers. comm.). In *Locusta* at least, this strand, or possibly, strands, connects to the hypodermis of the distal dorsal femur, and not to extensor muscle fibres. It does not appear to be capable of transmitting tibial movements to the FCO, but may have another role (Field submitted, and Pers. Obs.): as the tibia extends, tension on the main ligament is relaxed, until at full extension, all the fibres of the main ligament distal to the fine fibre are slack. If the fine fibre (termed the unloading strand by Field (submitted)) is now cut, the elasticity inherent in the FCO and attachment cells causes at least some of the fibres in the s strand to become stretched. The unloading strand therefore appears to be important in maintaining distalwards tension on the body of the mtFCO when the femur is extended (main ligament relaxed).

FEMORAL CHORDOTONAL ORGAN PHYSIOLOGY

Prothoracic and mesothoracic legs

Field and Pflüger (1989) have shown that the two scoloparia in the locust mesothoracic leg have different responses. The proximal scoloparium contains phasic, phasic-tonic, and tonic units. At least some of these cells respond most

strongly to low amplitude vibration (4 *u*m) of the receptor apodeme at 50-300 Hz. The overall firing frequency curve (tonic firing frequency plotted against the full range of leg angles) is unusual for a chordotonal organ because it is not U shaped: in this case, maximal firing occurred at full tibial extension, while minimal firing occurred at full flexion.

In contrast, the distal scoloparium (Burns 1974, Field & Pflüger 1989) contains phasic and phasic-tonic units which are most sensitive to low velocity, large amplitude apodeme movements (such as those which occur during walking). The overall firing frequency curve is U-shaped, with maximal firing frequencies at full tibial extension and flexion, and a minimal firing at mid-angles. Burns (1974) has shown for the distal scoloparium that individual neurones do not have maximal firing at both extremes of leg movement, and that the overall response observed must be a summation of the many different individual responses. This work was based on extracellular recordings, and was therefore rather limited. In particular, it was not always possible to be sure if only a single neurone was represented in any one recording. Ablation of the proximal scoloparium did not affect tibial reflexes known to be mediated by the FCO (Field & Pflüger 1989). The distal scoloparium however is known to mediate resistance reflexes in tibial muscles, and thus has a role in the control of posture (Field & Pflüger 1989).

It is interesting to note that a in a study of the FCOs of the bush cricket *Decticus*, Theophilidis (1986b) found maximal firing frequencies at full tibial flexion, a mid-angle minimum, and only a small increase in firing with further extension. Theophilidis notes that the sensitivity (change in firing frequency per change in femur-tibia angle) of the different FCOs in *Decticus*, and the prFCO and msFCO of *Locusta* are similar. *Decticus*' mtFCO has a higher sensitivity between 0 and 90° because of the mechanics of its apodeme's movement (Theophilidis 1986b).

Metathoracic leg

Both Usherwood et al. (1968) and Zill (1985a,b) have studied the responses of locust mtFCO neurones to tibial movements. Field & Burrows (1982) have investigated the reflex effects of the locust mtFCO on femoral muscles, while Field & Rind (1981) reported on intersegmental reflexes mediated by the mtFCO of the New Zealand weta (*Hemideina femorata*: Orthoptera). Several workers have undertaken research on the homologous organ in the stick insect (Bässler 1965, Hofmann & Koch 1985, Hofmann et al. 1985). The latter two papers

provide a detailed single-unit analysis, while Zill (1985a) provided a few intracellular recordings in the locust. For the locust in particular though, the available information is almost exclusively derived from extracellular records.

The pioneer study by Usherwood et al. (1968) revealed the characteristic U shaped firing frequency response curve (i.e., maximal firing at both extremes of leg movement), but these authors were unable to determine if different tonic units were active in different ranges of leg movement. Hysteresis and adaption of tonic mtFCO neurones were both reported in this early study. Phasic units fired during extension and flexion, with the former response being the stronger. Different units seemed to fire in response to movements in either direction. Phasic units were velocity sensitive, and adapted very rapidly. These authors also suggested that the mtFCO influenced reflexes in at least the tibial extensor and flexor muscles. Ablation experiments reported in the same paper suggested that because of its connections to the extensor and flexor tibiae muscles, the organ can monitor the "tonic state of the femoral muscles", thus providing information relevant to jumping (i.e., signal when sufficient tension has been developed).

Field & Burrows (1982) investigated the reflex effects of the mtFCO (but not details of the afferent response), and reported the following findings: (1) separate stimulation of the flexor strand or the main ligament could produce resistance reflexes in the extensor and flexor muscles; (2) these effects were strongest at leg angles where the stimulated strand was stretched (i.e., tibial extension for the FS, flexion for the main ligament); (3) there were inter-joint reflexes onto the depressor and levator tarsi motorneurones; (4) the strength of all reflexes was altered by stimulus velocity, the leg's set position, the initial direction of movement, and the activity of other neurones in the CNS; (5) slow motor neurones were activated mostly by slow stimuli, whereas fast motor neurones responded most strongly to rapid leg movements. It is interesting to note that the reflex effects mediated by the FS were much weaker than those produced by the main ligament. FS reflexes were not affected by the set position. In order to stimulate the FS, Field & Burrows (1982) moved the entire tibia (the apodeme had been cut, and was independently controlled). It is possible that some of the effects attributed to the FS were in fact due to passive movements of the flexor muscle apodeme (via vlig). In their defence, however, it should be noted that the 'flexor strand' effects were greatest at full tibial extension, when vlig is most relaxed and least likely to exert any influence on the mtFCO.

Zill's (1985a,b) studies of the locust mtFCO were the first attempt to describe the responses of individual receptors. His initial extracellular recordings confirmed the presence of hysteresis in the FCO output, and again suggested that the FS and main ligament activate different populations of sensory neurones. Zill (1985a) found no evidence to support Usherwood et al's (1968) speculation that the mtFCO can detect resisted contractions of the flexor muscle (i.e., it did not respond to muscle tension or distortion of the joint). His experiments however do not shed any light on the possible role of vlig, because he was only able to state that "chordotonal organ activity during spontaneous movements...was *similar* to that seen during imposed displacements..." (my italics). A more rigourous investigation of possible flexor muscle influences on mtFCO afferents is clearly required.

The intracellular recordings made by Zill (1985a) were largely made from neurone somata in the FCO. Because physiological stimuli move this region of the FCO, intracellular recordings are difficult to maintain. In practice, he could only be test stimuli of less than 15°. This limited what could be said about any neurone's complete response characteristics, including possible range fractionation. Zill attempted to overcome this by recording from the axons of 9 additional animals (1 neurone per animal ?), but he only presented a single figure representing 8 recordings. Considerable caution must be used when interpreting such a small dataset.

What is clear from Zill's (1985a) intracellular work is that: (1) individual phasic cells respond either to extension or flexion, but not to both; (2) the response of a unit was correlated to its position in the organ and its scolopidial orientation (flexion sensitive = dorsal, with scolopidium parallel to axis of femur; extension sensitive = ventral, with scolopidium at 30-35° to the axis); (3) some units fired single spikes only at the start of movements, and function simply as movement detectors; (4) all tonic receptors have maximal firing frequencies at one or other extreme of leg movement, and are inhibited at the opposite extreme; (5) all tonic units have a phasic component which produced an initial high firing rate in response to movement; (6) some tonic units are inhibited by one direction of movement (the opposite to that producing the phasic response). This inhibition may outlast the stimulus by up to a minute; (7) apart from 3 units which were located close to the dorsal attachment and responded to extension, all the tonic units followed the distribution pattern described above for phasic receptors; (8) some units responded to release of tension from either the FS or main ligament.

Zill (1985a) concluded that the FS stimulated neurones in the ventral group, while the main ligament stimulated dorsal somata. He also predicted that the adequate stimuli for each group should be different (i.e., longitudinal stress for dorsal cells with parallel scolopidia, and longitudinal stress and bending for the ventral cells with scolopidia at an angle to the axis of the femur). Zill (1985b) confirmed that leg reflexes are mediated by the FCO. He showed that when the tarsus was resting on a support, 'apparent movements' of the tibia (i.e., movements of the mtFCO apodeme) caused resistance reflexes in either the flexor or extensor tibiae motorneurone, depending on the direction of the stimulus. If the leg was not resting on a support, it was often being used by the animal to search for support. If the FCO was stimulated during this activity, resistance reflexes were not seen. Instead, any stimulus tended to excite the flexor and inhibit the extensor tibiae motorneurones ("flexor reflex mode". The gain and reliability of all reflex effects were quite variable, even between repeats of a stimulus within a single experiment. This plasticity is likely to be important in allowing the animal 'reflex-free' use of its leg during voluntary active movements such as walking. Zill (1985b) was unable to assign any function to the flexor reflex mode. In a later paper, Zill (1987) shows that in walking locusts, imposed stretch of the mtFCO apodeme consistently elicits resistance reflexes, which are maintained for the duration of the stimulus, regardless of its length. This strongly implies that the femoral chordotonal organ plays a role in load compensation.

CENTRAL PROCESSING OF FCO INFORMATION

Burrows' (1987) study of the central connections made by mtFCO afferents revealed a number of interesting results. He was able to show that some individual afferents connected monosynaptically to both motor neurones and spiking interneurones in a ventral midline group. Each afferent can synapse onto several motor neurones and interneurones, and conversely, each motor neurone or interneurone can receive inputs from more than one FCO afferent. Burrows (1987) did not attempt to characterise the FCO afferents before testing their connections, and therefore could say little about possible differences between the responses of different units. Similarly, he did not stain individual FCO afferent central projections. In a later study (Burrows 1988), further details of the connections to the ventral midline spiking interneurones were established. Some interneurones in this group are excited by tibial extension, others by flexion, and yet others by both directions of movement. An interneurone excited

by one direction of movement could either show no response to the opposite stimulus, or could be inhibited by it. Interneurones often had different responses in different arcs of leg movement (range fractionation). Both tonic (responding to static leg position) and phasic interneurones (responding solely to leg movement) were found. Some phasic interneurones only responded to movements within a certain velocity range, and were inhibited by all other movements.

Laurent & Burrows (1988) demonstrated monosynaptic connections of mtFCO afferents onto interneurones in a dorsal-lateral-anterior group which projected anteriorly in the connective (intersegmental interneurones). Individual interneurones responded to leg extension, flexion, or to both directions of movement with either tonic or phasic responses. Once again, FCO responses were recorded extracellularly by monitoring all the activity in the leg nerve, and selecting some units by the size of their spikes. This limited the characterisation to, at most, 'extension' or 'flexion' sensitivity.

Metathoracic femoral chordotonal organ neurones also make direct connections with nonspiking local interneurones (Burrows et al. 1988), which they excite. These same interneurones also receive inhibitory inputs from spiking local interneurones which are known to be directly excited by FCO afferents. The nonspiking interneurones can be phasically and/or tonically excited, and can respond to one or both directions of leg movement. These nonspiking interneurones make direct connections with leg motor neurones, which they control in sets, to bring about coordinated leg movements (Burrows 1980).

Figure 5 (modified from Burrows et al. 1988 and Burrows 1989) summarises the connections known to be made by locust mtFCO neurones. It is clear that information is transmitted in parallel to many interneurones and motor neurones. Direct connections to motorneurones could underlie *invariant* reflexes, while the more complex pathways provide a basis for the considerable *plasticity* needed during active behaviours.

Parallel research on stick insects

The intracellular studies made of stick insect FCOs (Hofmann & Koch 1985, Hofmann et al. 1985) are much more thorough than the comparable study by Zill (1985a). Hofmann and colleagues have catalogued the responses of many neurones to a range of carefully controlled stimuli in which position, velocity, and acceleration were manipulated independently. They reported the following types of neurones: (1) position sensitive; (2) velocity sensitive; (3) position and

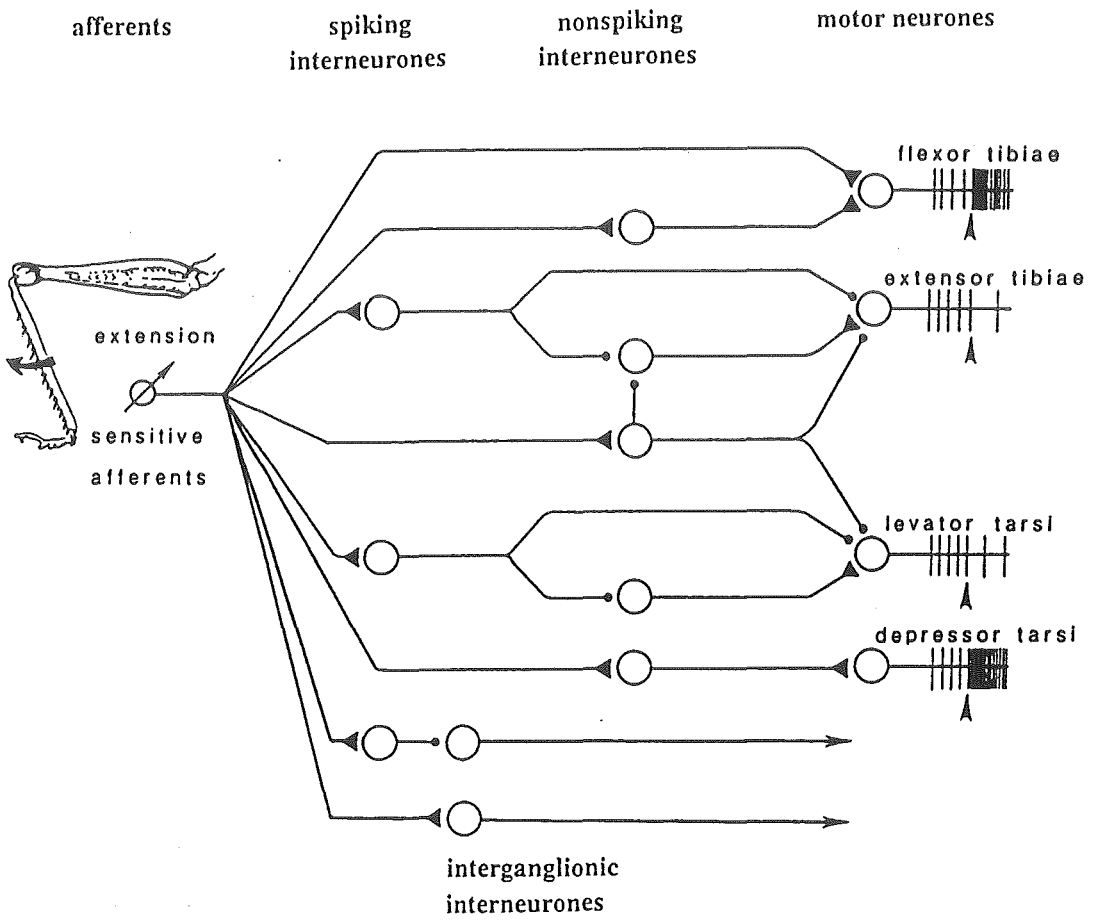


Figure 5. Summary of the central connections made by FCO neurones, and an example of their reflex output onto 2 groups of motorneurones in response to an extension of the tibia. Closed circles indicate inhibitory synapses, while triangles represent excitatory connections. Updated from Burrows et al. (1988) and Burrows (1989).

velocity sensitive; (4) acceleration sensitive; and (5) acceleration and velocity sensitive. There are graded boundaries between response types, with intermediate units in many cases. The responses of 'position and velocity' sensitive neurones in particular were often quite complex (Hofmann et al. 1985). Bässler (1988) has examined reflex effects of the stick insect FCO on various motor neurones, and is able to distinguish between at least 2 different types of reflex: (1) an 'active reaction', which controls the velocity of flexor muscle contraction; and (2) a typical resistance reflex which occurs in inactive animals. Debrodt & Bässler (1990) have shown that, in the stick insect, slow motor neurones are affected most strongly by slow movements of the FCO, while fast motor neurones respond best to rapid stimuli, thus confirming the results of Field & Burrows (1982) in the locust (although Debrodt & Bässler appear to be unaware of the earlier

work!). Debrodt & Bässler noted that although all the tested flexor motor neurones responded to stimulation of the FCO, they did not all receive exactly the same inputs. Büschges (1989) investigated spiking interneurones which receive inputs from the FCO, and was able to make several generalisations. However, his work was limited because of the relatively small range of leg angles used (at most the leg was moved between 70 and 110°), and because he was unable to say whether the connections were mono- or polysynaptic. He was unable to directly compare his results with similar work in the locust (Burrows 1988) because the accelerations of the stimuli applied to the FCO were not controlled in the latter work. However, Büschges stated that comparable interneurones did not seem to have been recorded in the 2 studies. Büschges' main findings (with brief comparisons to the results of Burrows 1988) are: (1) tonic position sensitivity was always accompanied by phasic responses to velocity and/or acceleration (in the locust some spiking interneurones responded only tonically); (2) velocity responses could be excitatory or inhibitory, and could be produced by extension or flexion, or by both directions of stimulus (also seen in the locust); (3) some velocity sensitive interneurones were also influenced by position; (4) acceleration responses were always excitatory, and often occurred in interneurones which did not respond to position or velocity; (5) convergence of FCO outputs onto single interneurones was suggested (as it has been in the locust (Burrows 1987).

THE OBJECTIVES OF THIS THESIS

There were clearly a number of questions about the locust mtFCO which required clarification. My supervisor, Dr Larry Field, had suspected for some time that there were more neurones in the organ than had been described by other authors. We decided that this fundamental question should be the starting point for my research. *My first goal was to redescribe the structure of the locust mtFCO, taking into account the probable existence of a large number of previously unrecognised neurones* (Chapter One).

Zill (1985a) had described the locations within the FCO of neurones with different responses (e.g., extension or flexion sensitivity). I felt that it was important to expand this by mapping for the first time the specific locations of characterised somata. Zill's characterisation of neurones was limited because he did not cover the full range of leg angles used by the animal. I overcame this problem by recording from the axons rather than the somata of FCO neurones.

It was hoped that the responses of the newly described distal neurones (Chapter One) would be included in this analysis. *My second goal was therefore to compile a map of neurone somata locations in the organ, and relate the location of each neurone to details of its response to a full range of tibial movements* (Chapter Two).

Some sensory afferent neurones are known to project into the CNS in an ordered way related to their response or their location on the body surface (tonotopic or topographic mapping). An understanding of such mapping is an important prerequisite to studies of the connections made by sensory neurones. Although some of the central connections of FCO neurones had been demonstrated by previous workers (see above), a study of the underlying anatomical organisation of sensory afferents was clearly lacking. *My third main goal was therefore to investigate the central branching patterns of characterised FCO neurones* (Chapter Four).

Zill's (1985a) work on the locust mtFCO, and Hofmann & colleagues' investigations of the homologous organ in the stick insect had shown that different neurones responded in different ranges of joint angle (range fractionation). These authors had also noted the presence of hysteresis in the responses of tonic neurones (i.e., neurones fired differently at a given angle depending on whether the angle had been approached by an extension or a flexion movement). Both these phenomena must be clearly understood before it is possible to interpret how the CNS is able to extract meaningful information from the complex patterns of sensory input. Zill's report did not include many response types that I had recorded (Chapters Two & Four), while the stick insect research failed to present a thorough description of the overlap between FCO neurone's response ranges. The large dataset that I had compiled during the experiments described in Chapters Two and Four provided me with an ideal opportunity to investigate the extent to which range fractionation and hysteresis occurred in the FCO. *My third goal was to provide the first specific study of hysteresis, and range fractionation of both tonic and phasic neurones in the FCO* (Chapter Three).

ORGANISATION OF THE THESIS

Each of the chapters in my thesis is essentially a complete manuscript in itself, and therefore has its own Introduction and Discussion. The first 2 chapters have already been published. Chapter One is included here unmodified (although its layout has been changed to match the style of the thesis). Chapter Two was written and published soon after I had completed my investigation of cell body

locations and responses. During the subsequent investigation of central branching (when the distal somata could not be stained), I recorded from some FCO neurones with responses different to those described in Chapter Two. I have added this information into Chapter Two for the sake of completeness. This involved adding some new figures, updating the table, and making some textual alterations.

In order to minimise overlap, all the references have been compiled into a separate section at the end of the thesis. Some additional material (largely details of methods used, and a listing of the computer program that I designed to run the ramp generator) has been included in the appendices.

Although the first chapter is co-authored, the division of labour is quite clear-cut: Larry Field (my supervisor) suggested the investigation of FCO structure because his preliminary cobalt backfills revealed neurone-like structures in the main FCO ligament. Larry's fills however were not conclusive, and neither axons or dendrites could be confirmed. Thus, all the results presented in the Chapter (except Fig. 1a and 6b,c) came from my own work. I wrote the text and prepared the figures (except Fig. 6). As with all the chapters, Larry provided many critical comments on the text and figures. H.J. Pflüger (Chapter One) and anonymous referees made a number of comments about the first two chapters which enabled me to make some improvements.

All the other work in this thesis is solely my own. Larry and I have discussed this work many times, and I gratefully acknowledge his considerable influence on my understanding of this subject in particular, and of neurophysiology as a whole. Any misconceptions and errors, however, can only be attributed to my own shortcomings.

CHAPTER ONE

Innervation of the metathoracic femoral chordotonal organ of *Locusta migratoria*

Thomas Matheson and Laurence H. Field

[Published in *Cell & Tissue Research* (1990) 259: 551-560]

SUMMARY

The locust metathoracic femoral chordotonal organ is the largest sense organ in the hind leg. It has been intensively studied, and used as a model input system in studies of central integration of proprioceptive information. We have used electron microscopy, as well as whole nerve and single axon cobalt backfilling to show that previous descriptions of the metathoracic femoral chordotonal organ have failed to recognize a substantial group of small neurons distal to the known cell bodies. This distal group increases the number of neurons known to be present in the organ from a previous maximum of 55 to an average of 92. The axons of the distal neurons form a bundle separate from those of the proximal cells, suggesting that the distal neuron group is the homologue of the distinct proximal scoloparium of the pro- and mesothoracic femoral chordotonal organs. In the light of these findings several earlier studies of locust neurobiology should be re-examined. Future investigations also need to recognize the complexity of the metathoracic femoral chordotonal organ.

INTRODUCTION

In strand chordotonal organs, the neuron dendrites insert singly or in pairs into scolopidia, which are complex ciliated mechanotransducer structures embedded in the elastic strand which spans the joint (Young 1970; Füller and Ernst 1973). The largest sense organ in the locust hind leg is the femoral chordotonal organ (FCO). This is a receptor which spans the femur-tibia joint, monitoring static position, and the direction, velocity, and acceleration of movement of the tibia (Hofmann and Koch 1985; Zill 1985a). The FCO controls resistance and assistance reflexes, and catalepsy behaviour in the stick insect (Bässler 1983). It is one of the most intensively studied insect proprioceptors (Hubbard 1959; Usherwood et al. 1968; Burns 1974; Field and Rind 1981; Field and Burrows 1982; Bässler 1983; Hofmann *et al.* 1985; Zill 1985a,b; Theophilidis 1986a,b; Burrows 1988; Field and Pflüger 1989). In addition, the FCO has recently been used as a model chordotonal organ in studies of central integration pathways of proprioceptors (Laurent 1986; Burrows 1987, 1988; Burrows *et al.* 1988; Büschges 1989).

Although the metathoracic FCO (mtFCO) has been known for some time, early descriptions dealt mostly with its gross morphology (Eggers 1928; Debaissieux 1938) or development (Slifer 1935). The number of sensory neurons in the mtFCO, as determined from histological sections, has varied with different authors. Slifer (1935) counted 55 in the grasshopper *Romalea*. In 1968 Usherwood *et al.* published a light micrograph of the organ in the locust *Schistocerca*, and counted approximately 24 cell bodies. Recently several other authors have also attempted to determine the number of neurons in the mtFCO. Zill (1985a) reported 45-55 neurons, while Theophilidis (1986a) noted 55-60 neurons in *Decticus* (a tettigoniid orthopteran); both authors used light microscopy on histological sections. Studies using axonal backfilling with cobalt chloride usually yielded fewer neurons. Grosch *et al.* (1985) and Burrows (1987) reported 35 and 29-44 cells, respectively, while Pflüger *et al.* (1988) reported 20-30 cells. We had similar results until we modified the filling regimen for cobalt infusion.

We describe in this paper a previously unreported cluster of neurons in the mtFCO. They greatly increase the known cell complement of this sense organ. Our results are confirmed by cobalt hexammine nerve backfills, intracellular injection of cobalt into single neurons, and transmission electron microscopy of the mtFCO and its nerve. The implications of these results are discussed with regard to current research on the role of the mtFCO, and with regard to homologies between the mtFCO and the FCO's of the pro- and mesothoracic legs of the locust.

MATERIALS AND METHODS

Adult locusts (*Locusta migratoria*) of both sexes obtained from our laboratory culture were used for all experiments. Figure 1a was obtained from *Schistocerca gregaria americana* during an early phase of this study, conducted in Australia (by LHF).

Cobalt staining

Twenty seven animals were used for cobalt backfilling of the chordotonal organ. Locusts were held dorsal side down on a plasticene platform by strips of plasticene placed over the forelegs and over the abdomen. The metathoracic femora were rotated and held with plasticene so that their anterior surfaces faced upwards. Nerve 5b1 was exposed in mid-femur and the distal cut end placed in a vaseline cup containing distilled water. After 10-30 s the water was replaced with a 5%

solution of hexamminecobalt (III) chloride (Sigma). The vaseline cup was then sealed, and the animal placed in a moist chamber in a refrigerator (4°C) for 3-7 days. After removing the animal from the refrigerator the vaseline was scraped away, and the cuticle overlying the chordotonal organ was carefully dissected off. The leg was removed from the body and placed in 3-4 ml of saline to which 3 drops of concentrated ammonium sulphide were added. Cobalt sulphide was precipitated for 10-15 minutes. The leg was then washed in saline, fixed in Carnoy fixative, dehydrated in an ethanol series, and cleared in methyl salicylate. The chordotonal organ was then removed from the leg, viewed and photographed using a Leitz Orthoplan microscope with differential interference contrast optics. Chordotonal organs were also drawn onto a standard grid with the aid of an eyepiece graticule.

Locusts restrained in the same way were used for intracellular dye injection into individual chordotonal organ neuron somata. A small window of cuticle overlying the chordotonal organ was removed, and a grooved silver support platform manoeuvred under the organ. Drops of locust saline (pH 6.8) were added if required to prevent drying out of the preparation. Microelectrodes (100-130 MΩ) were filled with a saturated solution of hexamminecobalt (III) chloride. Up to 4 neurons per chordotonal organ (but usually only 1) were penetrated and filled. Filling took 10-15 min using 10 nA, 200 ms pulses at 2 Hz. Chordotonal organs were dissected out immediately, and processed as described above, before rehydration, silver intensification (Bacon and Altman 1977) and re-examination. Dye leakage or non-specific staining was not a problem. In a few cases, however, the scolopale cell associated with a stained neuron also showed signs of staining when intensified and viewed with interference contrast optics.

TEM examinations

Entire chordotonal organs from 4 animals, with a short length of n5B1 still attached, were dissected out and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 8 hr at 4°C before being washed overnight in buffer (4°C). Organs were then postfixed in 1% osmium tetroxide buffered as above for 2-4 hr at 4°C before a further overnight wash in buffer (4°C). The chordotonal organs were then dehydrated in an ethanol series, transferred to acetone, and embedded in Spurr's low viscosity resin. Ultrathin sections were cut on an LKB 8800 ultrotome using glass knives. Sections were stained in 3%

uranyl acetate, followed by lead citrate, and then viewed with a Jeol 1200EX TEM at an accelerating voltage of 80kV.

RESULTS

Our early attempts to infuse the mtFCO with cobalt chloride via n5B1 revealed a number of proximal neurons (soma diameters 10-50 *u*m) and a group of small (7-15 *u*m) faintly stained ovoid or round structures further distal (Fig. 1A). By increasing the filling time from 3 to 7 days, and using hexamminecobalt chloride instead of cobalt chloride it became evident that the distal structures were the nuclei of small neurons (Fig. 1B,C). These neurons possessed typical scolopidial structures which characterize chordotonal organ neurons (Fig. 1C). The cell bodies occurred up to 250 *u*m distal from the positions of previously described chordotonal organ neurons. Figures 1A and 2A show how these cells are arranged in a distally-tapering pattern, concentrated towards the ventral margin of the chordotonal organ's distal strand. Many separate collagenous fibres along the dorsal margin form the distal attachment of the FCO to its apodeme. The axons leading to the distal neurons could not be seen in backfilled preparations (presumably because they were obscured by the large numbers of darkly stained somata), although sometimes dendrites were evident. Cobalt backfills showed that the mtFCO contains on average 92 neurons (standard deviation = 6.99, range = 76-107, n = 27).

In order to demonstrate the presence of axons, distal neurons in 45 preparations were dye-filled using microelectrodes (Figs 1C,1D). Their axons were clearly visible travelling proximally from their somata, over the chordotonal organ, and eventually joining into the cuticular nerve and n5B1.

Ultrathin sections cut from chordotonal organs at locations A-F (Fig. 2B), and examined with the TEM, confirmed the presence of neuron somata and scolopidia more than 200 *u*m distal from the previously described neurons. The longest dendrites of proximal cells do not extend this far within the strand (Fig. 1B, inset), so these distalmost scolopidia must be associated with the distal somata. In addition, individual scolopidia could be followed in sequential sections to their respective neuron somata and axons (Fig. 3A-F). There were no differences other than neuron size between distal and proximal neurons. Neurons always inserted in pairs into scolopidia, as found in other insect chordotonal organs (Young 1970; Slifer and Sekhon 1975). A section (Fig. 3A) cut through plane A (Fig 2B) reveals the distal-most scolopale cap (SC) in this chordotonal organ. At

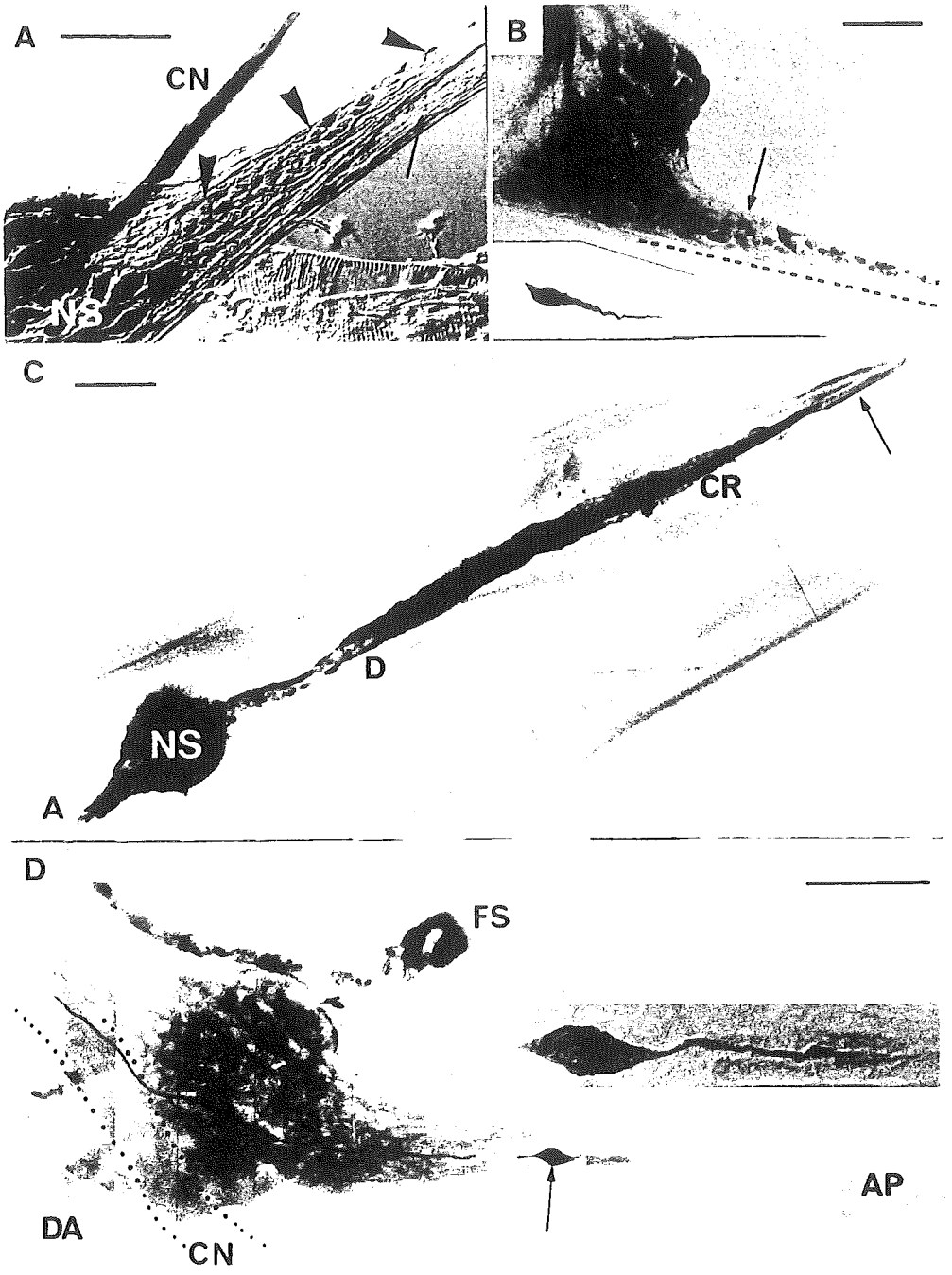


Figure 1 A-D. Distal neurons of the locust mtFCO. (A) Interference contrast photomicrograph of a cobalt filled mtFCO. The distal neurons are barely visible (*large arrowheads*), due to lack of cobalt filling. The somata (*NS*) of large proximal neurons are well filled. Many parallel collagenous strands (*small arrow*) form the distal attachment of the chordotonal organ to its apodeme. The cuticular nerve (*CN*) has been displaced from its usual dorsal path during tissue preparation (cf. Figs 1d,2a). (B) A more darkly stained preparation reveals many distal neuron somata (*Dashed line*). The inset (at the same magnification) shows one of the largest proximal neurons (from a different preparation). (C) Close examination of an intracellularly stained distal neuron (soma at location indicated by arrow in B, but from a different preparation) shows a dendrite (*D*) containing darkly stained ciliary root (*CR*), ending in scolopidium (*arrow*). The neuron soma (13 um dia.) (*NS*) and axon (*A*) are also clearly visible. (D) Injection of cobalt into a distal neuron soma (*arrow* and *inset*) reveals the path followed by its axon, which joins the cuticular nerve (*CN, dotted outline*). *FS* flexor strand; *AP* collagenous strands leading to apodeme; *DA* dorsal attachment. *Scale bars*: A,B,D = 100 um; C = 10 um.

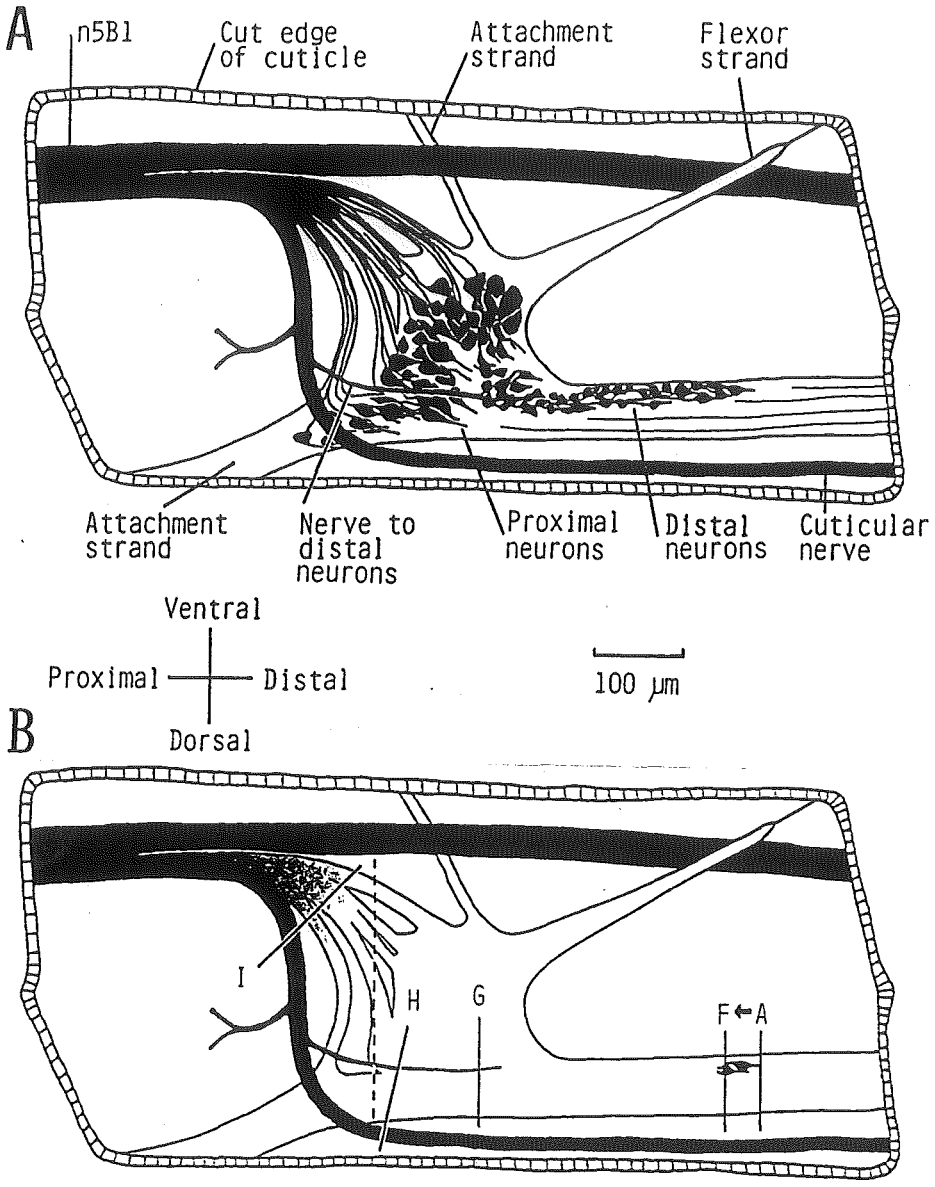


Figure 2 A,B. (A) Drawing showing the innervation of the mtFCO revealed by cobalt backfilling (viewed through a window cut in the anterior side of the distal femur). Axons from the distal somata form a small nerve which joins the cuticular nerve distal to its junction with axons from proximal neurons. (B) TEM sections for Figs 3 and 4 were cut from a chordotonal organ at locations *A-I*. The reference line used for distance measurements is shown (*dashed line*).

this level only one of the two cilia is present (arrowhead). Microtubules are prominent in the surrounding attachment cell (AtC). A section cut through the same scolopidium further proximally (Fig. 3B) reveals paired dendrites (D_1,D_2) surrounded by 9 scolopale rods (SR) and a scolopale cell (S). Proximal to the scolopale rods (Fig. 3C) the dendrites dilate (cf. Ball 1981) while still enclosed in the scolopale cell (S). In Fig. 3D the second-distalmost scolopidium appears (D_3,D_4, and associated structures as given in Fig. 3B), close to the original pair of

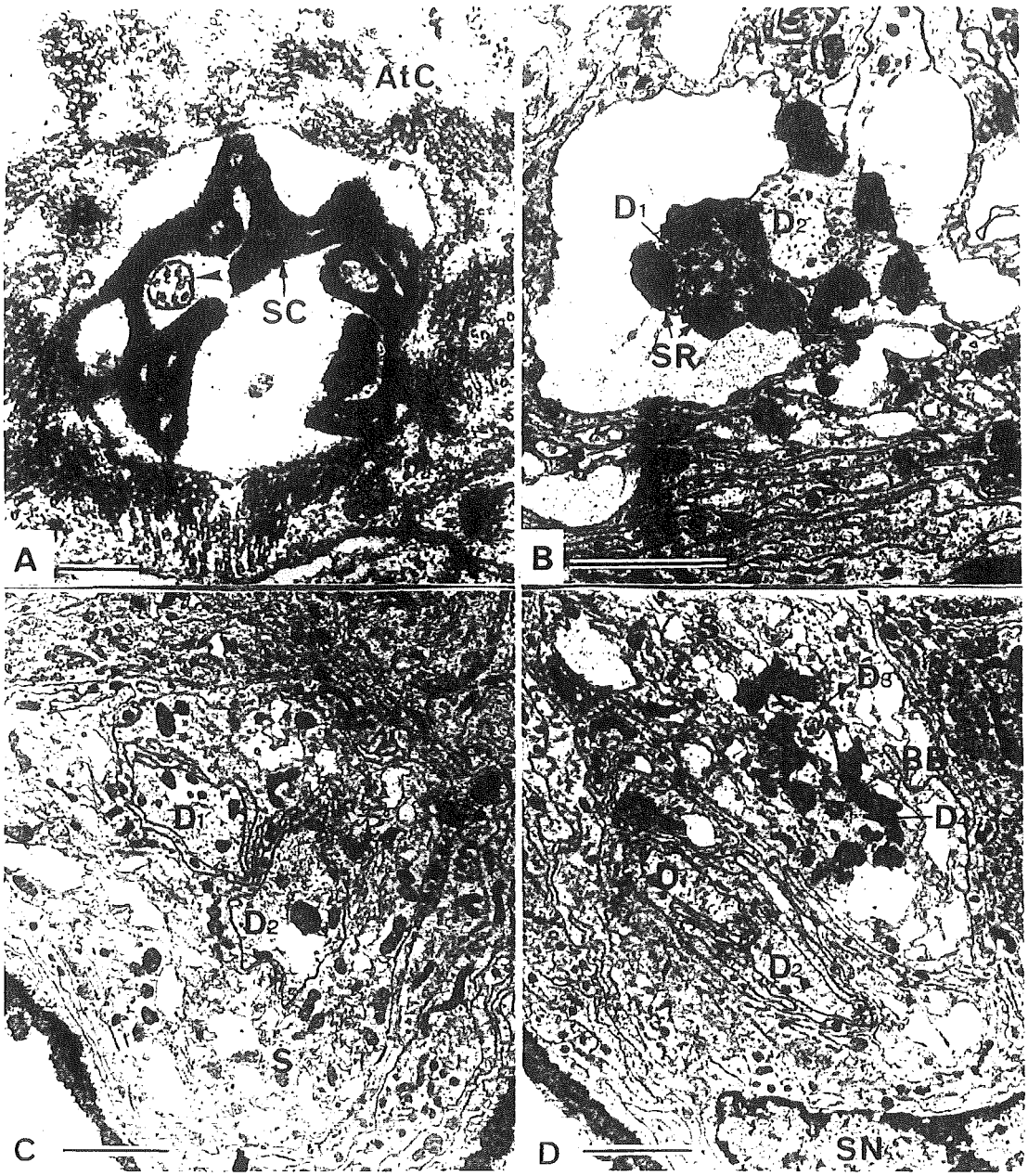
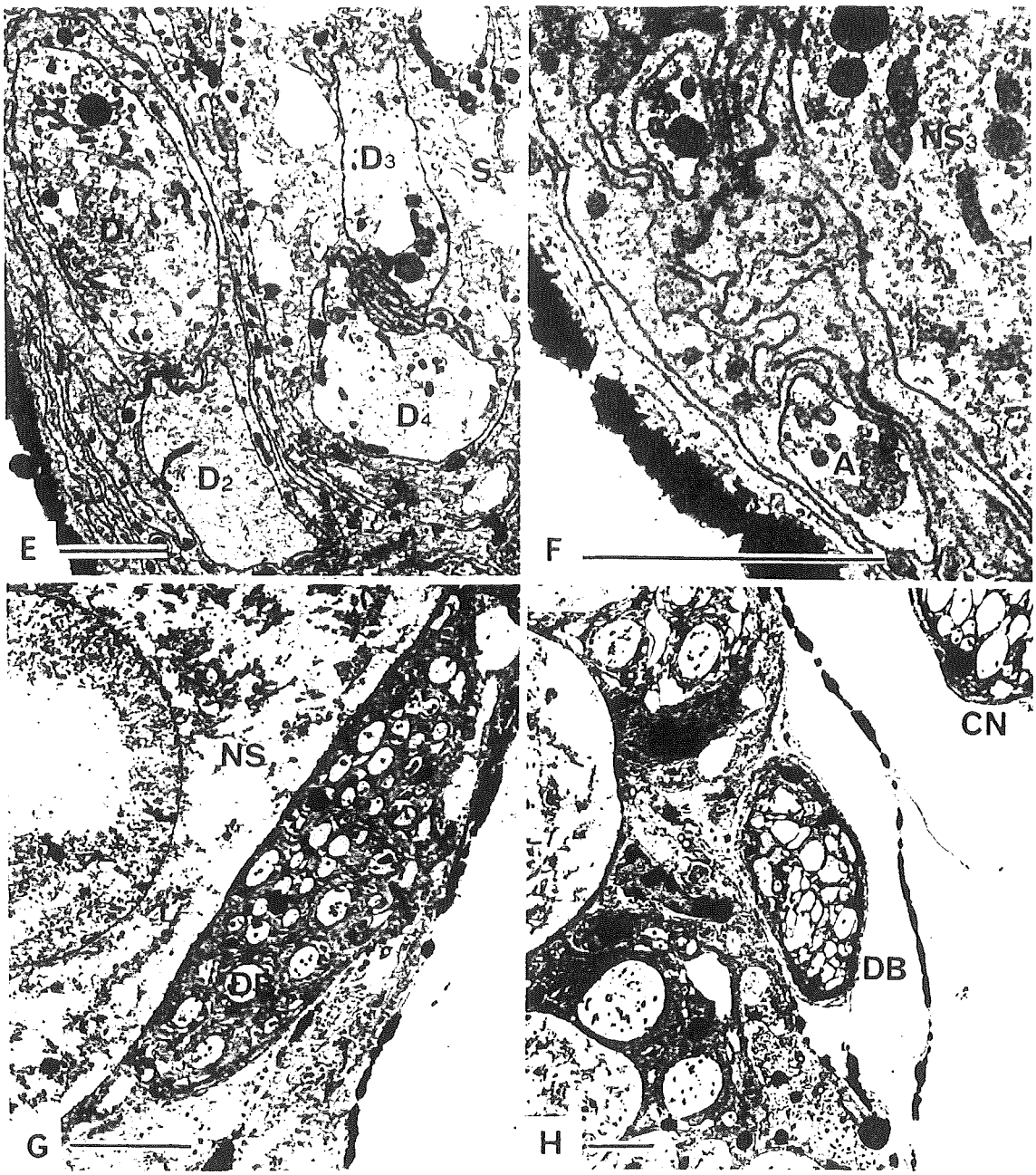


Figure 3 A-H. Non-consecutive serial sections cut through one chordotonal organ (*locations A-I in Fig 2b*). (**A**) A section through the scolopale cap (*SC*) of the most distal pair of neurons (*plane A of Fig. 2b*). One of the two sensory cilia is present at this level (*arrowhead*). The attachment cell (*AtC*) is densely populated with microtubules. (**B**) The dendrites (D_1,D_2),are surrounded by scolopale rods (*SR*) and a scolopale cell (*S*). (**C**) Proximal to the scolopale rods the dendrites continue to be surrounded by the scolopale cell (*S*). (**D**) The first pair of dendrites (D_1,D_2) are still present at the level of the second-distalmost scolopidium (D_3,D_4 and associated structures as given in **B**). D_4 has been cut through at the level of a basal body (*BB*). A scolopale cell nucleus (*SN*) is visible at the bottom of the figure. (**E**) Both pairs of dendrites (D_1,D_2 and D_3,D_4) are present here. D_1 is beginning to widen as it approaches its soma. (**F**) The axons of the 2 distalmost neurones (A_1,A_2) lie close to the darkly stained outer margin of the chordotonal organ in this section cut proximal to the first pair of neuron somata. The soma of one of the second-distalmost pair of neurons is visible (NS_3). (**G**) The axons from many distal neurons form a compact bundle (*DB*

Continued overpage...



...*Continued from previous page*

distal bundle) just under the surface of the chordotonal organ. The soma of a large proximal neuron (*NS*) with spherical nucleus also appears. (**H**) The distal axon bundle (*DB*) has almost become superficial to the chordotonal organ in this section. It soon joins the cuticular nerve (*CN*) which lies close to the outer surface of the chordotonal organ (see Fig. 2a). *Scale bars*: **A** = 0.2 *u*m; **B-F** = 2 *u*m, **G,H** = 5 *u*m.

dendrites (D_1,D_2). D_4 has been sectioned at the level of a basal body (BB) (Ball 1981). A scolopale cell nucleus (SN) is also present. Eventually (Fig. 3E) the first pair of dendrites (D_1,D_2) begin to widen as they approach their respective somata. D_3 and D_4 are surrounded by a scolopale cell (S). There is often close

contact between dendrites (e.g., D_1,D_2). The functional significance of this (if any) has not been pursued. A section cut proximal to the first pair of somata (Fig. 3F) reveals their axons (A_1,A_2) lying just below the surface of the chordotonal organ, and the soma of another distal neuron (NS_3). A section through plane G of Fig. 2B reveals the tightly clustered bundle of axons (DB = distal bundle) from many distal neurons. This bundle of axons maintains its position close to the surface of the chordotonal organ as it travels proximally. Eventually the bundle becomes superficial (Fig. 3H) and later (Fig. 4) joins the small nerve (CN) which passes over the main body of the chordotonal organ (Fig. 2A). This nerve (the *cuticular nerve*) also contains the axons of small hair sensilla located further distal in the femur (Zill 1985a). Axons from the proximal group of neurons join the cuticular nerve in a number of loose bundles several hundred microns further proximal (Figs 2A, 4). At this level (plane I of Fig. 2B) the bundle of axons from the distal group is present as a cluster of small diameter glia-wrapped profiles on the side of the cuticular nerve nearest to the chordotonal organ (DB in Fig. 4). A similar-looking bundle of about 10 axons on the opposite side of the cuticular nerve in this particular preparation may have arisen from proximal-dorsal somata, but could not be followed in subsequent sections. The remainder of the axons in the cuticular nerve (presumably all from hair sensilla) lack the thick glial wrapping.

The diameters of axons from proximal and distal neurons were measured from electron micrographs of sections from 4 animals (Table 1). Axons with diameters greater than 2.6 *u*m were not found in the distal neuron bundle, whereas proximal neurons had axons up to 5.1 *u*m in diameter. The smallest axons in both groups were the same size (0.3 *u*m). The total number of axons per chordotonal organ (range 94-110) corresponds well with the number of somata counted in our cobalt backfills.

A single axon (arrow in Fig. 4) which did not merge into the chordotonal organ may lead to a multi-terminal receptor known to branch from the cuticular nerve proximal to the chordotonal organ (L.H. Field, unpublished).

The size distributions of mtFCO neuron somata were determined for 6 complete cobalt backfilled organs. These results were averaged and plotted topographically in Fig. 5A. An arbitrary reference point was chosen to be the most proximal cell of the ventral group of proximal neurons (dashed line in Fig. 2B). In 2 of the 6 organs used for the size distribution analysis some dorsally located neurons lay proximal to the reference point, resulting in negative position values. The smallest neuron measured by Burrows (1987) was 15 *u*m in diameter.

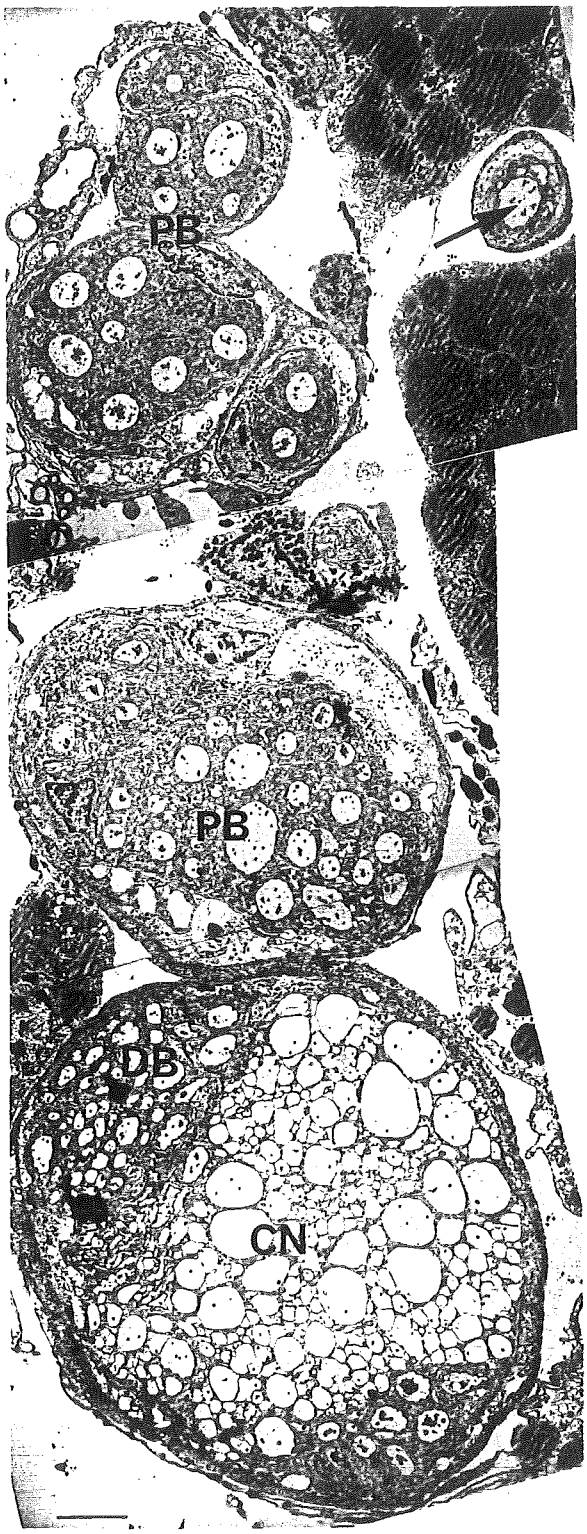


Figure 4. A composite electron micrograph taken at position *I* of Fig. 2b shows: 1/ loosely grouped bundles of axons from proximal neurons (*PB*); 2/ the distal bundle (*DB*) completely fused into the cuticular nerve (*CN*); 3/ a single axon (*arrow*) possibly of the multiterminal receptor associated with the mtFCO. Note the dense glial wrappings around the chordotonal organ axons, contrasting with the lack of glia around most other axons in the cuticular nerve. The glia wrapped axons at the lower right could not be traced to the chordotonal organ, so their destination is unknown. *Scale bar*: 5 *u*m

Table 1. Summary of axon sizes and numbers derived from TEM examination of 4 animals' chordotonal organs. Axons were measured from sections just distal to their separation from n5B1 (for proximal axons) or from the cuticular nerve (for distal axons). Numbers in brackets are the total number of neurons in each chordotonal organ.

Animal	Number of axons		Mean diameter (*u*m)		Range (*u*m)	
	Proximal	Distal	Proximal	Distal	Proximal	Distal
1[a]	--	30 (--)	--	0.8	--	0.4-1.6
2	47	47 (94)	2.0	0.8	0.3-5.1	0.3-1.7
3[b]	36	44	2.1	1.0	0.3-4.1	0.5-2.0
	--	30(110)	--	0.8	--	0.3-2.6
4	49	48 (97)	0.8	0.9	0.3-1.8	0.3-1.8
Summary	44	50(100)	1.6	0.9	0.3-5.1	0.3-2.6

[a] It was not possible to measure the axons from the proximal group because no successful sections were cut in this region.
[b] In this preparation the axons from the distal group formed two distinct bundles which joined the cuticular nerve separately.

We found many smaller neurons, particularly in the distal group of cells. Our results showed an average of 1.3 neurons per chordotonal organ of 5-10 *u*m diameter, and 32 per chordotonal organ of 10-15 *u*m diameter. These classes of cells occurred throughout the organ and up to 580 *u*m distal to the reference point (Fig. 5A). Neurons greater than 25 *u*m in diameter occurred only in the proximal group (i.e., < 200 *u*m from the reference point). Thus in the L-shaped distribution of Fig. 5A, the left hand leg represents the proximal group, while the right hand leg represents the newly described distal group of small cells. For comparison we have plotted in Fig. 5B the distribution of 33 mtFCO neurones which could be accurately measured from Burrows' (1987) illustration. Here the most distal cell was approximately 230 *u*m from the reference point, emphasizing that his staining showed only the proximal group. No cells larger than 35 *u*m were illustrated, although he reported neurons up to 70 *u*m in diameter.

DISCUSSION

Number of cells

Our results approximately double the number of sensory neurons reported for the mtFCO. Recent illustrations (e.g., Burrows 1987) of the mtFCO only show

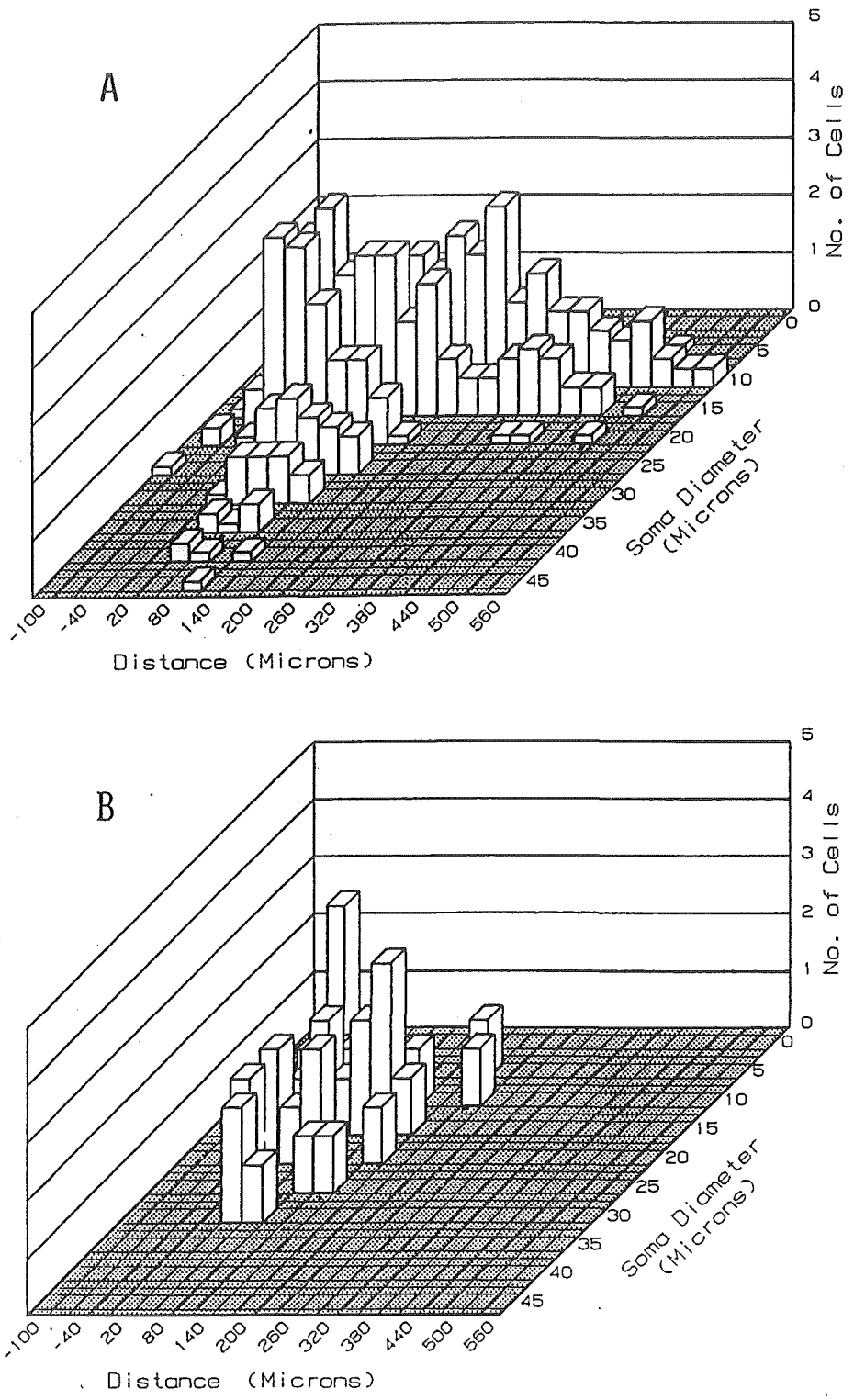


Figure 5 A,B. Three-dimensional plots of the sizes and spatial distribution of neuron somata in the chordotonal organ. (A) Mean frequencies from 6 animals. The distal group of small neurons is clearly represented in the right-hand leg of this plot. Larger neurons are only present closer to the reference point (*vertical dashed line in Fig 2b*). Most of the bars representing neurones in the 5-10 *u*m size class are obscured. (B) A similar plot of neurons measured from an earlier published illustration (Burrows 1987) lacks the distal group.

proximal neurons, most of which are large (Fig. 5B). It is likely that the small distal neurons did not stain with cobalt because of their tiny axonal diameters, which average 0.8-1.0 *um* (Table 1). Some small proximal neurons also may not have filled. Because we noticed faint stains of cells in the distal region of the mtFCO with conventional cobalt staining (Fig. 1A), and earlier, with methylene blue vital staining, we persisted in our attempts to determine whether these were indeed neurons. The prolonged cobalt backfilling regimen allowed the distal cells to take up the dye in visible concentrations after 7 days, but this approached the limits of maintaining a viable preparation under refrigeration.

The FCO's of the pro- and mesothoracic legs (prFCO, msFCO) contain two distinct groups of neurons: the proximal and distal scoloparia. The proximal scoloparium is a cluster of separately innervated tiny neurons often partially or completely isolated from the larger cells of the distal scoloparium (Field and Pflüger 1989). On the basis of the smaller number of cells that he observed, Burns (1974) suggested that the mtFCO has probably lost the proximal scoloparium evolutionarily, in parallel with the modification of the metathoracic leg into a jumping leg. In Fig. 6 the mtFCO (Fig. 6A) is compared with that of the New Zealand weta (Orthoptera, Stenopelmatidae) (Fig. 6B), which has partially fused scoloparia, and with the locust msFCO (Fig. 6C) which has separate scoloparia. The common feature in all of these is the separately innervated group of small cells, which forms the proximal scoloparium of the latter two chordotonal organs. We suggest that the newly described cells in the locust mtFCO are homologous to the proximal scoloparium, and that the distinction between scoloparia is more accurately determined by innervation than proximal or distal locus. Pflüger *et al.* (1988) suggested that the mtFCO proximal scoloparium is lacking, a conclusion based upon the central projection pattern of the whole organ. However their conclusion is weakened by the high probability that the distal bundle axons did not fill centrally because of their small diameters and the great distance from the filling site to the ganglion (Pflüger, pers. comm.). Unfortunately, the crucial experiment which would resolve the proximal / distal scoloparium question (showing the central projections of distal cells) appears technically difficult. The very short length of nerve available for backfilling (see Fig. 2), plus the problems cited above, argue against achieving successful central projection backfills of the distal cells without contamination from proximal cells.

It would be of interest to know whether the proximal and distal scoloparia have different physiological attributes in all cases. Field and Pflüger (1989) examined this question for the msFCO and found that the distal scoloparium

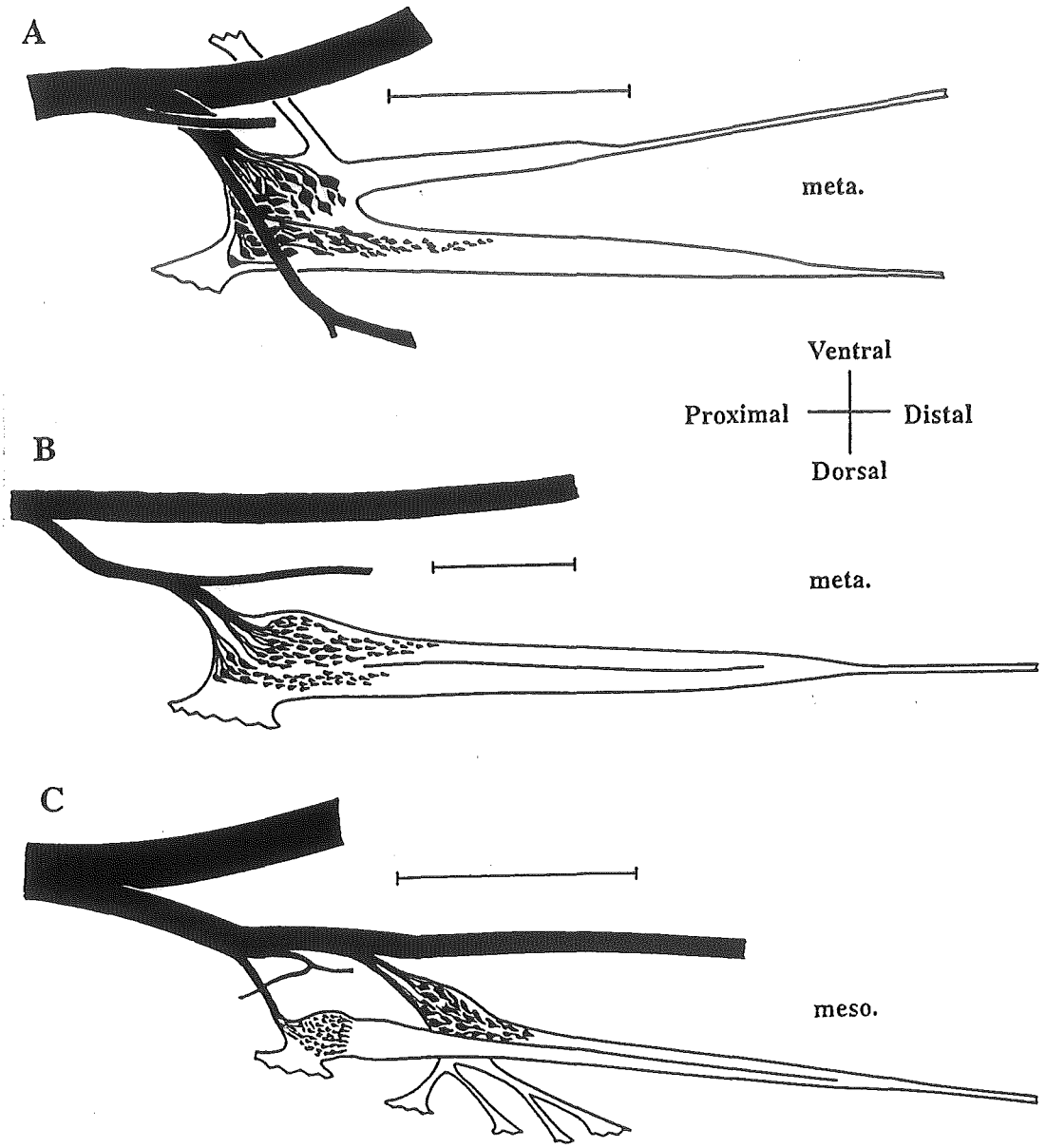


Figure 6 A-C. Differing degrees of fusion between the proximal and distal scoloparia are apparent in locust mtFCO (A), New Zealand weta mtFCO (B) and locust msFCO (C). In all three cases the two groups of neurons form distinct axon bundles. *Scale bars*: A = 500 um; B,C = 1000 um.

mediated the postural resistance reflexes, whereas the proximal scoloparium appeared to be highly vibration sensitive and did not alter the resistance reflex when it was removed. Attempts to record from, and stain single mtFCO distal neurons while stimulating the mtFCO (Matheson 1990), have so far proved refractory (presumably because of the small axon diameters).

It is now clear that the mtFCO is a more complicated sense organ than previously thought. Not only does it have twice the complement of sensory

neurons, but it also has additional sensory structures associated with it. The flexor strand, which connects the mtFCO to the flexor muscle apodeme (Field and Burrows 1982), contains a single strand receptor (Bräunig 1985). It is extension sensitive and is separately innervated via n3B5c by a central cell body. An undescribed multi-terminal receptor axon merges with the mtFCO nerve at the base of the chordotonal organ (L.H. Field, unpublished), as does the cuticular nerve (Slifer 1935), which branches to hair sensilla on the distal femur (Zill 1985a). As a consequence of this complexity, attempts to determine central projections of the mtFCO afferents (Burrows 1987; Pflüger *et al.* 1988) may well have contamination from other receptors. Both Pflüger *et al.* (1988) and Field and Pflüger (1989) noted the possibility of tactile hair axons in their central FCO projections. The complexity of receptor modalities in the mtFCO nerve also interferes with the conclusions of Macmillan and Kien (1983) who attempted to isolate the effects of chordotonal organ afference on walking coordination by electrically stimulating the mtFCO locus through the cuticle with wire electrodes. It is now apparent that in such experiments specific localization of current is necessary, but probably difficult to achieve.

Implications for FCO research

Burns (1974) suggested that the mtFCO gave considerably less detailed information on the position of the tibia than the prFCO or msFCO because of its apparently smaller number of cells. Our results imply that the mtFCO has sufficient neurons to provide very detailed information about tibial position. Indeed, current research by one of us indicates that mtFCO afferents code complex information about the position, and direction, velocity, and acceleration of movements of the tibia (Matheson 1990). Another problem related to a lack of understanding of the correct neuronal complement of the mtFCO arose in the study of Lutz and Tyrer (1988). They were led to the indirect conclusion that single mtFCO neurons are immunoreactive to both anti-cholineacetyltransferase and anti-serotonin, by the reasoning that the populations reacting to each antibody numbered 30-40 cells, and that the total number of cells was only 55. Our results do not negate the possibility that some neurons are immunoreactive to both substances, but the reasoning used by Lutz and Tyrer (1988) is no longer valid.

Current research on central afferent integration pathways in the locust requires accurate knowledge of the innervation of the mtFCO. Both spiking and non spiking interneurons have been shown to receive inputs from FCO afferents

(Burrows 1987, 1988; Burrows *et al.* 1988), but the identity of the afferents is unknown. Burrows (1987) and Pflüger *et al.* (1988) showed that individual mtFCO afferents project to different neuropilar areas in the metathoracic ganglion, but it is not known if the differing branching patterns are accompanied by differences in physiological responses. The next stage of research would be to specify the relationships between physiologically and morphologically identified afferents and their central projection patterns onto sensory interneurons.

CHAPTER TWO

Responses and locations of neurones in the locust metathoracic femoral chordotonal organ

Thomas Matheson

[Published in *Journal of Comparative Physiology* (1990) 166: 915-927]

SUMMARY

Insect legs possess chordotonal organs which monitor leg angle, and the direction, velocity and acceleration of leg movements. The locust metathoracic femoral chordotonal organ (mtFCO) has previously been studied morphologically and physiologically, but no detailed analysis of the responses of individual neurones, and their location in the organ has so far been produced. By recording from, and staining mtFCO neurones I have been able to compile for the first time such a map.

The distribution of neurone somata in the locust mtFCO is more complex than previously thought: receptors sensitive to both stretch and relaxation of the apodeme are distributed throughout the organ. The positions of neurones from seventeen response types were mapped within the mtFCO. Neurones with a particular response type have somata in comparable locations. Comparisons are made between the response types found in the stick insect and those in the locust. The possible functions of some of the responses are discussed.

INTRODUCTION

The femoral chordotonal organs (FCOs) of insect legs monitor femur-tibia angles, and respond to movements of this joint (locust: Usherwood et al. 1968; Burns 1974; Zill 1985a; *Decticus*: Theophilidis 1986; stick insect: Bässler 1965; Hofmann et al. 1985). FCO afferents make connections to motorneurones, spiking interneurones, and non-spiking interneurones in the thoracic ganglia (Burrows 1988; Burrows et al. 1988; Büschges 1989), and play a part in controlling leg reflexes, posture, and walking (Bässler 1976; Field and Rind 1981; Field and Burrows 1982; Zill 1985b, 1987; Zill and Jepson-Innes 1988). The proximal scoloparium of the locust mesothoracic FCO may be primarily a vibration receptor (Field and Pflüger 1989).

Locusts' metathoracic legs are modified for jumping and kicking (Heitler and Burrows 1977). Burns (1974) suggested that associated with this, the metathoracic FCO (mtFCO) has no homologue of the proximal scoloparium found in both pro- and mesothoracic FCOs, and this "means that the CNS must receive

considerably less detailed data on the positions of the metathoracic tibiae than on the positions of the other tibiae". Matheson and Field (1990) have shown histologically that the mtFCO possesses a group of neurones which probably represents the proximal scoloparium, raising the possibility that the mtFCO provides the same quality and quantity of information to the CNS as do the pro- and meso- FCOs.

To date, only three studies have attempted a comprehensive intracellular survey of the responses of individual FCO afferents (Hofmann and Koch 1985; Hofmann et al. 1985; Zill 1985a). All other studies have concentrated on extracellular recordings from either the chordotonal organ nerve, or nerve $5B_1$, which contains FCO (and other) afferent axons on their way to the metathoracic ganglion (Usherwood et al. 1968; Burns 1974; Field and Burrows 1982; Theophilidis 1986; Burrows 1988). Zill (1985a) recorded intracellularly from the somata of mtFCO neurones, but was unable to move the tibia through its full arc without pulling the recording electrode from the cell body. He thus provided only a limited assessment of the responses of individual receptors. Zill (1985a) also recorded from mtFCO axons in 9 animals in an attempt to overcome this problem, but did not provide a complete explanation of the response types found. Hofmann and Koch (1985) and Hofmann et al. (1985) thoroughly examined the responses of many individual FCO neurones in the stick insect *Cuniculina impigra*, and grouped them into 17 classes. Their study has allowed other authors to go on to make accurate investigations of the central processing of stick insect FCO afference (Büschges 1989), a task not yet possible in the locust.

The present study of locust mtFCO afferents is important in three respects: it allows accurate comparisons with the stick insect work; it greatly extends the information available on locust FCO afferent responses (with direct relevance to any study of central integration pathways); and for the first time it provides a detailed map of locations of mtFCO neurone somata with known responses.

As noted in the General Introduction of the thesis, this chapter has been updated since its publication. The additional information (derived from a separate study of the central projections of mtFCO neurones) concerns response types only, because the peripheral cell bodies were not stained. This information has been merged into the results section, and Table 1 has been modified. There are some minor changes to the discussion. This approach was considered to be clearer than the alternative, which was to present an addendum including the new results, an updated table and a re-discussion of the findings.

In the results section, unless specifically stated otherwise, numbers of neurones refer only to neurones which were mapped (i.e., any additional observations from the central projection study are mentioned separately). Table 1, however, contains the total number in each class, derived from both studies.

MATERIALS AND METHODS

Adult locusts (*Locusta migratoria*) of both sexes obtained from our laboratory culture were used for all experiments. Only left hind legs were used. The results presented in this paper are from 76 successful recordings. Additional results (from the central projection study) are derived from 113 other animals (see Chapter Four for methods).

Locusts were held dorsal side down on a plasticene platform by strips of plasticene placed over the forelegs and over the abdomen. The metathoracic femora were rotated and held with plasticene so that their anterior surfaces faced upwards; the femur-tibia joints were fixed at 60°. In this paper the term 'leg angle' refers to the femur-tibia (F-T) angle (Fig. 1). A small area of cuticle overlying the metathoracic ganglion was cut away, allowing nerves 3 and 5 to be severed close to the ganglion. This procedure precluded muscle contractions in the leg under study, thereby stopping the animal from fracturing and distorting the weakened femur-tibia joint. The thoracic opening was sealed with vaseline.

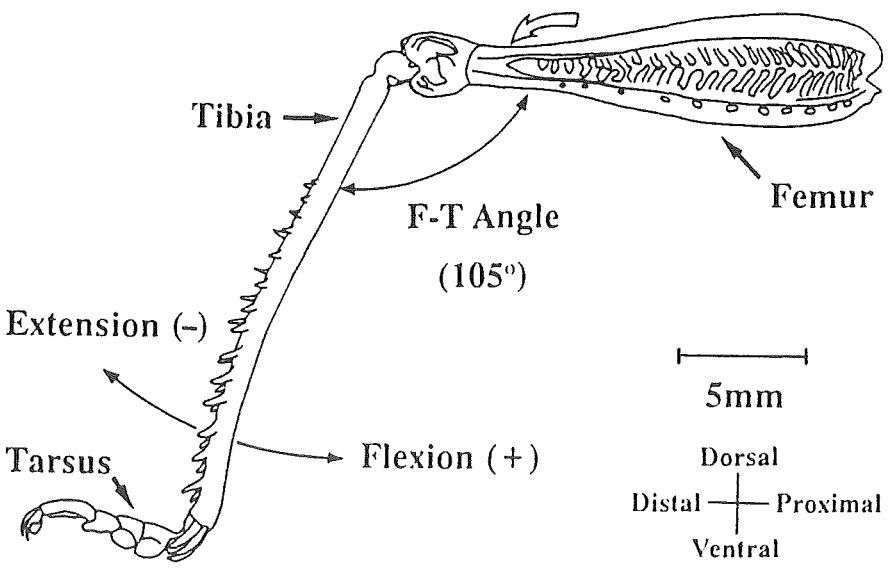


Figure 1. Locust metathoracic leg held at 105°. The main segments are labelled, and the approximate position of the FCO under the dorsal cuticle is indicated (open arrow). Extension (-) and flexion (+) movements are shown.

The apodeme of the FCO was exposed in the distal femur and grasped by handmade miniature titanium forceps attached to a Ling V101 vibrator. Drops of locust saline (pH 6.8) were added if required to prevent drying out of the preparation. The apodeme was cut distal to the forceps. A custom built analog ramp generator under computer control moved the apodeme to mimic tibial movements between 0 and 120°. (The range of possible femur-tibia angles is 0-150°, but other than during kicking, animals rarely extend their legs beyond 100° (M. Coles unpublished). During normal walking the metathoracic femur-tibia angle oscillates within the range 40-90° (Burns 1973)). A 175 *u*m movement of the apodeme was equivalent to a 20° movement of the tibia in *Locusta*. Stretching the apodeme mimicked tibial flexion, while relaxation mimicked extension. In this paper, apodeme movements are referred to as either extensions or flexions. The velocity and direction, but not acceleration of movements were controlled. The flexor strand (Field and Burrows 1982) was left attached to the tibia, and remained set at 60° throughout all manipulations.

Nerve 5B1 was exposed in mid-femur through a separate window 3-5 mm from the FCO (thereby leaving intact the accessory flexor muscle which inserts onto the cuticle close to the FCO). A groove cut in a wide earthed silver platform positioned under the nerve provided great stability for intracellular recording. Movements of the apodeme did not cause any perceptible movement of the leg nerve at this distant recording site.

Microelectrodes (100-130 MΩ) filled with a saturated solution of hexamminecobalt (III) chloride were mounted on a Leitz micromanipulator and used to impale individual axons in n5B1. When a stable intracellular recording was obtained, the neurone's responses to a variety of apodeme movements were recorded on a Teac MR-10 recorder. Action potential height varied greatly between recordings (as also reported by Hofmann et al. 1985), but most recordings were quite stable, allowing unambiguous staining of single neurones. On a few occasions more than one neurone took up dye, but these preparations were discarded. After characterising the neurone it was filled with cobalt for 15-45 min using 400 ms pulses at 1.5 Hz. Following dye-filling, some cuticle and overlying tissues were removed from around the chordotonal organ, taking care not to disturb its various attachment points (Usherwood et al. 1968; Zill 1985a). The distal part of the tibia, and the proximal femur were removed before the remainder of the leg was immersed in 5 ml of saline containing three drops of concentrated ammonium sulphide. Cobalt sulphide was precipitated for 10-15

minutes. The leg was then washed in saline, fixed in Carnoy's fixative, and dehydrated in an ethanol series. During fixation both the flexor strand and the apodeme were held at approximately 90°. The chordotonal organ was then removed from the leg, rehydrated, silver intensified (Bacon and Altman 1977) and examined using a Leitz Orthoplan microscope with differential interference contrast optics. Chordotonal organs were photographed, and filled neurones drawn onto a standard outline.

RESULTS

Classification of receptors

Twenty four response types were recorded from neurones in the metathoracic femoral chordotonal organ. Table 1 summarises the main types: other distinctions are made in the text. It is possible that other responses are also present, but were not encountered. In broad terms, individual units responded to: (1) tibial velocity; (2) tibial acceleration; (3) position and velocity; (4) position and acceleration; or (4) to all three parameters. No purely tonic (position sensitive) neurones were found. Units were usually directionally sensitive (i.e., they responded to either extension or flexion, but not to both). The only bidirectionally responsive units were some acceleration receptors (see next paragraph and Discussion). Some neurones had their greatest response at maximum extension or flexion, while others responded most strongly near the mid-point of the tibia's movement. It should be borne in mind that these experiments utilised movement of the apodeme alone, with the flexor strand fixed at 60°. Thus neurones responding solely to movements of the flexor strand (i.e., in ranges of joint extension (Zill 1985a)), will not be found. This will tend to bias the relative numbers of flexion versus extension sensitive neurones recorded (as seen in Table 1). Previous studies (Field and Burrows 1982; Zill 1985a) suggest that few, if any, individual neurones respond to both flexor strand and apodeme movements, so the movement protocol used in my study is unlikely to distort neurone responses. In addition, midpoint-tension was maintained on the flexor strand during apodeme movements, further reducing the possibility of artifacts. Direct manipulation of the apodeme (unlike movement of the entire tibia) allows stimulation of the FCO through wide arcs of movement (0-120° in a single step) while maintaining accuracy for small movements, and is thus the preferred stimulus.

Some neurones appeared to be acceleration sensitive because they responded with 1-4 rapid spikes (usually 1) at the start and end of ramps (cf.

Table 1. Locust metathoracic femoral chordotonal organ neurone response types. Numbers in brackets represent neurones which had unclear responses, or stained very poorly. Also shown are corresponding values from Hofmann et al. (1988). Asterisks indicate response types recorded from the central projection study only. Note that values in this table represent the total number of neurones of each response type, derived from both my studies (i.e., including the central projection results). In the text, values usually refer only to *mapped* neurones (unless stated otherwise).

Response type	Number recorded	
	My study	Hofmann et al.
Position-and acceleration		
$P^0?A$ [a]	6 (1)	11 (2) [c]
$P^{60}?A$ [a]	7 (1)	3 (1) [bc]
$P^{120}?A$ [a]	0	3 [c]
Velocity		
V^+	33	12 (1)
V^-	18 (2)	10
V^{+-}	0	8 (2)
Acceleration		
$?A^+$	8	0 (1)
$?A^-$	4	12 (3)
$?A^{+-}$	2*	16 (7)
?AS	17	
Position-and-Velocity		
P^0V^+	17 (1)	13 (5)
P^0V^-	1	1 (2)
$P^{60}V^+$	5*	0 [b]
$P^{60}V^-$	0	0 [b]
$P^{120}V^+$	0	2
$P^{120}V^-$	7 (1)	3 (2)
Velocity-and-acceleration		
$V^+?A^+$	2*	0
$V^+?A^-$	0	2 (3)
$V^-?A^+$	0	0
$V^-?A^-$	16 (1)	4 (1)
$V^+?A^{+-}$	0	1
$V^{+-}?A^{+-}$	0	5
Position-velocity-and-acceleration		
$P^0V^-?A^-$	1	0
$P^{60}V^-?A^-$	16 (2)	0
$P^{120}V^-?A^-$	4 (2)	0
$P^0V^+?A^+$	4*	0
$P^{60}V^+?A^+$	4*	0
$P^{120}V^+?A^+$	0	0
$P^{20}V^+?A^-$	1*	0

[a] Acceleration types (A^+, A^-, A^{+-}, AS) have not been differentiated.

[b] Units classified as P^{mid} by Hofmann et al. (1985) also have a velocity dependent component, and probably correspond to my $P^{60}V^+$ and $P^{60}V^-$ types.

[c] Position sensitive units recorded by Hofmann et al. (1985) do not have an acceleration component.

Hofmann and Koch 1985, and see Discussion). For ease of description the term acceleration receptor (?A) has been used, although proof of acceleration sensitivity is tentative because ramp accelerations were not controlled independently from position and velocity. A subset of acceleration receptors responded in a similar way, but fired only at the start of ramps in both directions, and not at the end of ramps. I therefore suggest that they are designated ?AS (for 'acceleration at the start' - also see Discussion).

Abbreviations for the different response types follow those used by Hofmann et al. (1985). All neurones with a tonic discharge coded for position (i.e., the firing frequency differed at different set angles). These neurones were labelled 'P'. The angle causing the maximum tonic firing rate was added as a superscript (e.g., P^{70}, P^{0}). If there was hysteresis in the response, then the two curves were averaged prior to determining this angle. Neurones which responded to velocity (V) or start of movement (?AS) were given a superscript '+', '-' or '+-' to indicate directional sensitivity (Fig. 1). A '+' (flexion) or '-' (extension) code indicates unidirectional sensitivity whereas a '+-' code means that the neurone responded to movements in both directions. Putative acceleration receptors (?A) were given a superscript '+' or '-' to indicate the sign of the acceleration to which they responded ('+' accelerations occur at the start of flexion (+) movements, and at the end of extension (-) movements, while '-' accelerations occur at the end of flexion (+) and start of extension (-) movements).

When a neurone responded to more than 1 parameter of a movement the appropriate abbreviations were combined. For example, a neurone having a tonic discharge maximum at 70°, and responding to flexion movements in a velocity dependent way was represented as $P^{70}V^{+}$.

Velocity receptors

Twenty-two neurones which responded solely to the velocity of movements were encountered (Table 1). V^{+} receptors fell into two groups: ventral and dorsal (Fig. 2a,b). Those in the ventral group, near the flexor strand insertion (Fig. 2a), fired much more strongly during flexions as the leg angle nearly reached 0° (full flexion). These neurones only responded to velocities greater than $40°s^{-1}$. At velocities of $40\text{-}200°s^{-1}$, often the only response was a single spike as the leg flexed from 20° to 0°. At higher velocities ($400\text{-}1000°s^{-1}$) spikes occurred during earlier flexions (i.e., at more extended angles) (Fig. 3a), and the spike frequency increased (Fig. 3a, insets). Figure 4 shows (for 1 V^{+} neurone) how joint angle

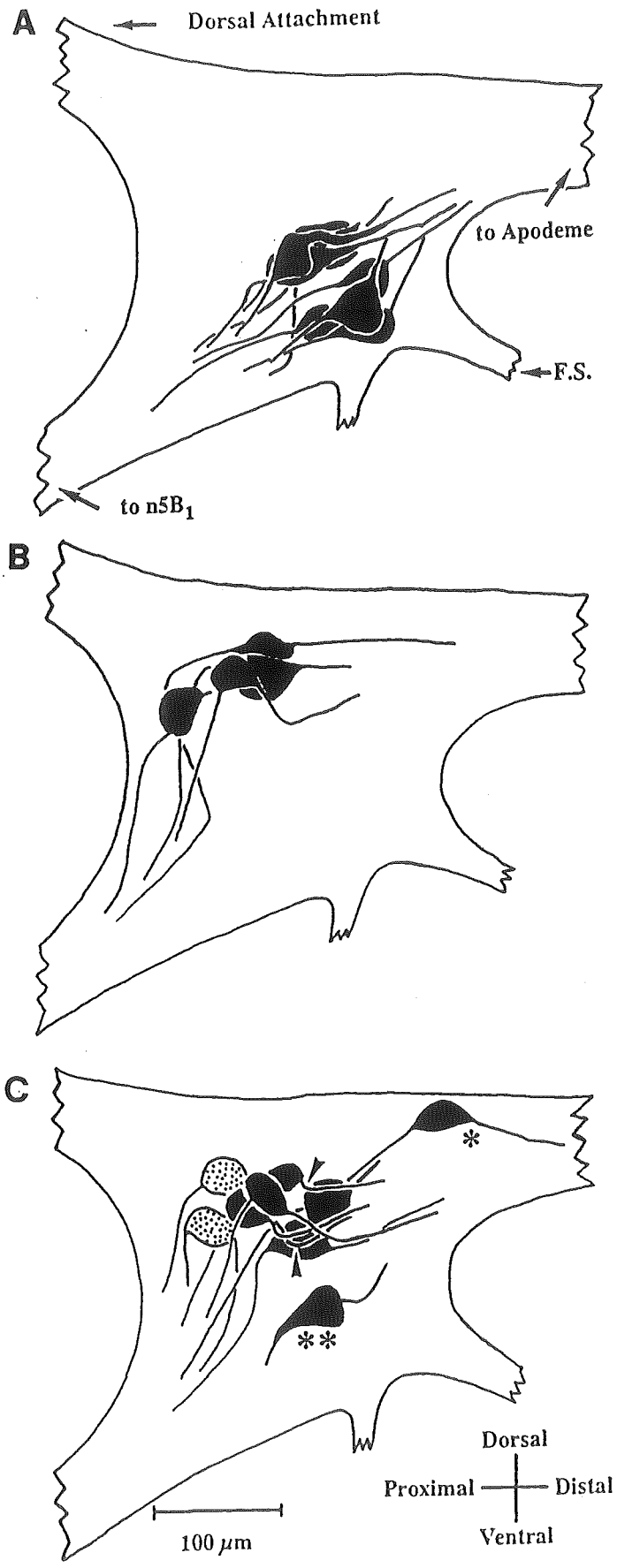


Figure 2 A-C. Velocity sensitive neurones' locations in the mtFCO. (A) V^+ neurones in this ventral group fired most strongly as the leg approached 0°. (B) V^+ neurones in this dorsal group fired in response to leg flexions in a wide range of leg angles. (C) V^- neurones fell into 1 central group with 2 exceptions (*asterisks*). *Arrowheads* indicate pronounced bends in the dendrites of some of these cells. Stippled cells were poorly filled, and may not be accurately represented.

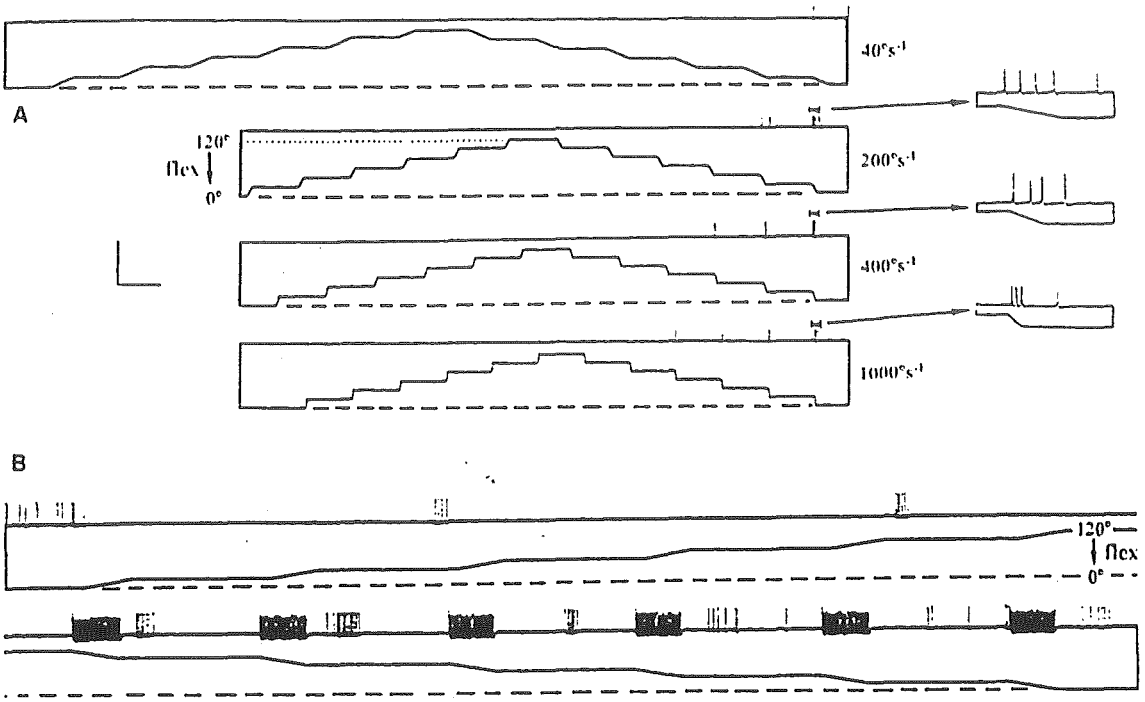


Figure 3 A,B. Responses of velocity sensitive (V^+) neurones to 20° movements. (A) V^+ neurone from the ventral group: higher velocities caused spikes to occur at more extended angles, and increased the firing frequency (*insets*). (B) V^+ neurone from the dorsal group: flexions at $20°s^{-1}$ elicit high frequency firing throughout the leg's arc of movement. Other bursts of spikes are caused by movements of tracheae near the mtFCO. *Scale*: vertical; A = 20 mV, B = 18 mV: horizontal; A,B = 1 s, insets = 78 ms.

determines the firing frequency during movements at different velocities.

In contrast to V^+ neurones in the ventral group, V^+ neurones in the dorsal cluster (Fig. 2b) fired strongly during flexions over a wide range of leg angles (Fig. 3b). Neurones in this group were very sensitive, responding to movements as slow as $0.5°s^{-1}$ with intense bursts of spikes. As with V^+ neurones in the ventral cluster, increasing the velocity of leg movement caused spikes to appear during earlier flexions (more extended angles).

V^- receptors (Table 1) were found in one central group (Fig. 2c), with two exceptions (* and ** in Fig. 2c). The distal, dorsal cell (*) responded to movements as slow as $6°s^{-1}$ with a strong barrage of spikes, whereas other V^- receptors only began to respond between $40\text{-}200°s^{-1}$ (and even then, with only a few spikes per ramp). Unlike other V^- receptors, which fired most strongly during extensions close to 120°, the distal one fired more strongly during extensions close to 0°. One V^- receptor located ventrally with respect to the central group (** in Fig 2c) also fired more strongly close to 0°, but in other respects (spike frequency,

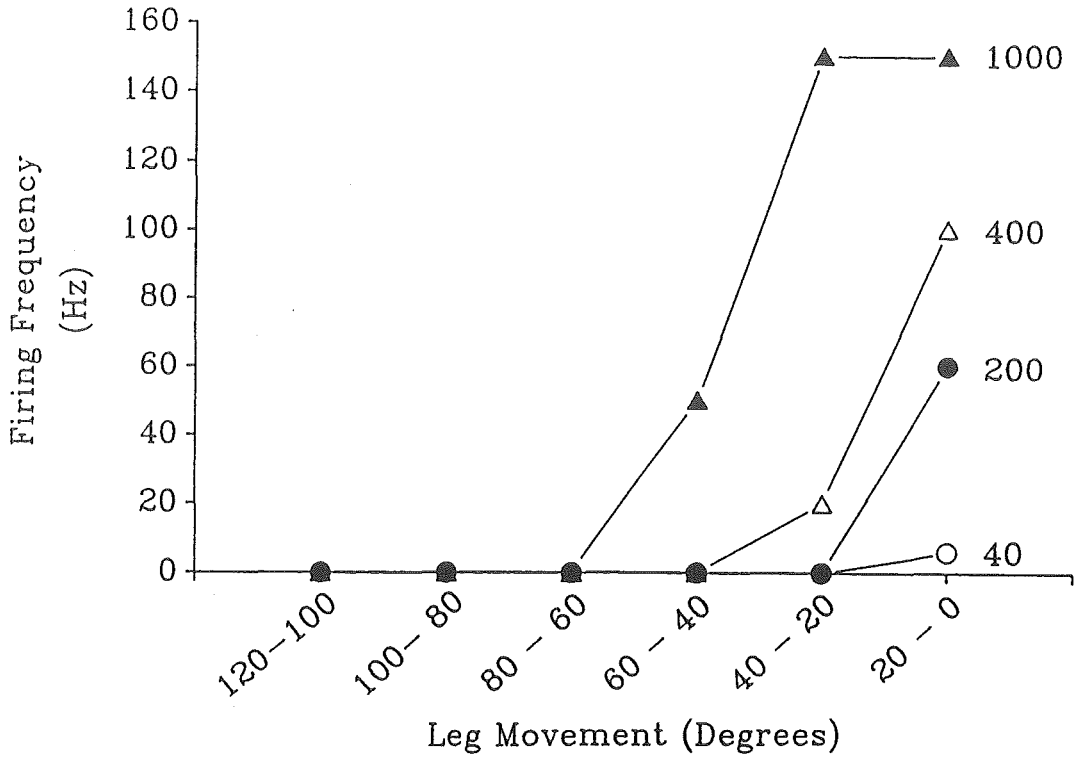


Figure 4. The effect of velocity on the angle at which spikes occur, and on the spike frequency of a V^+ neurone from the ventral cluster. Numbers associated with each line are the ramp velocity ($°s^{-1}$).

sensitivity) it resembled the majority of V- receptors.

The dendrites of 4 of the 7 V^- neurones in the central group, and the ventral neurone (** in Fig. 2c) had a conspicuous angle (e.g., arrowheads in Fig. 2c). This seems to be a real feature of these neurones because there was no apparent distortion of FCO.

Acceleration receptors

Two $?A^+$ (Fig. 5a) and one $?A^-$ receptor (Fig. 5b) were filled (Table 1). All three were found in the same location. The $?A^+$ units responded with 1-4 spikes (usually 1) to the start of flexions, and to the end of extensions, while the $?A^-$ receptor fired at the end of flexions and the start of extensions. Both of the $?A^+$ receptors responded more strongly (more spikes per ramp, and fewer spike failures) during V^+ ramps than during V^- ones. The direction of the movement had no effect on the response of the $?A^-$ neurone.

Six acceleration sensitive units which responded with 1-2 spikes (usually 1) to the start only of both flexion and extension movements (?AS) were also encountered. Their location in the FCO (Fig. 5c) was similar to that of the $?A^+$ and $?A^-$ receptors. Two of these 6 neurones responded more strongly to V^-

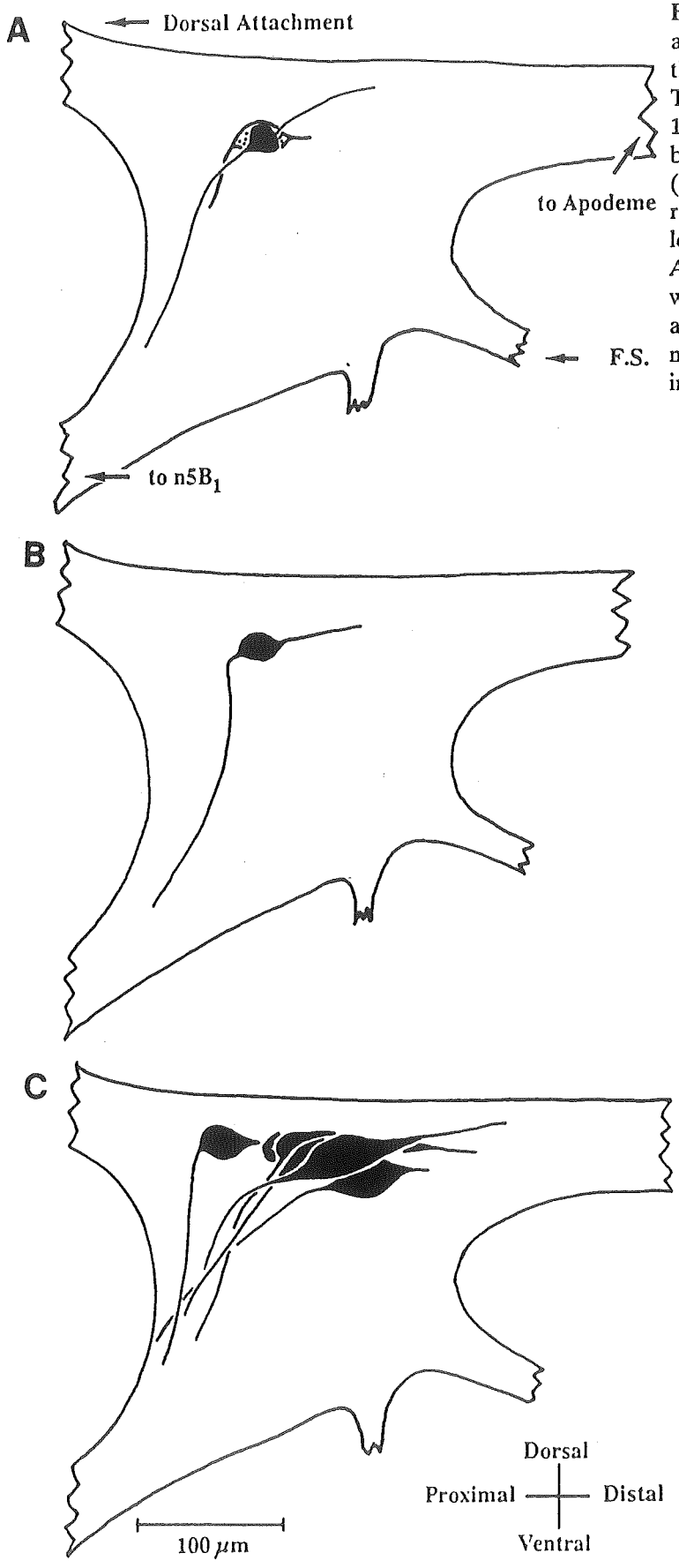


Figure 5 A-C. Locations of acceleration sensitive neurones in the mtFCO. (A) ?A$^+$ receptors. The stippled cell occasionally fired 1 spike at the start of the extension between 0-20° (?A$^-$ type response) (B) ?A$^-$ receptor. The only ?A$^-$ receptor stained was in the same location as the 2 ?A$^+$ cells. (C) Acceleration sensitive neurones which only responded to accelerations at the start of movements (?AS) were all located in this dorsal group.

movements than to V^+ movements. One neurone responded more strongly to V^+ movements, while the remaining 3 were not influenced by the sign (direction) of the velocity.

Increasing the velocity of the stimulus did not consistently affect the number or frequency of spikes fired by acceleration sensitive (?A or ?AS) receptors, but it often increased the range of angles over which spikes occurred. At high velocities (e.g., $400°s^{-1}$) acceleration sensitive receptors fired during movements at all angles (0-120°). When near their threshold, some acceleration receptors began to lose responsiveness during movements close to 120°.

During the central projection study two neurones were recorded which responded with 1 or 2 spikes at the start and end of movements ($?A^{+-}$ response: Fig. 6). Unlike ?AS receptors which fire only at the start of movements in either direction, these $?A^{+-}$ receptors often fired equally strongly at the start and end. The neurone represented in Fig. 6 only fired at the end of flexion movements between 60° and 0°. It did not fire at the end of any extension movements. The second $?A^{+-}$ neurone fired at the start and end of movements in both directions, and across the full range of leg angles.

Position-and-acceleration receptors

Four neurones (Table 1) located close to the dorsal attachment point of the organ (Fig. 7) responded to static leg angle, and to acceleration. The tonic firing rates of 2 of these increased as the leg approached 0° (P^0) (Fig 8a,b), while the others' maxima were at 60° (P^{60}) (Fig. 8c,d). Firing was completely or partially inhibited during movements in either direction (Fig. 9a). There was no evidence of post-inhibitory rebound. The firing frequency of one P^{60} neurone changed only slightly at different leg angles across most of the leg's arc of travel, but had a marked change between 60-100° (Figs 8d,9b). This occurred to a lesser extent in one P^0 receptor (Fig. 8b,9a). These neurones are therefore more sensitive to changes in position between 60-100° than to similar magnitude movements in other parts of the leg's arc. Other position sensitive neurones showed graded changes in firing frequency across their entire response ranges (e.g., Fig. 8a,c). The acceleration component of many of these neurones was often hidden by the high level of tonic discharge, only becoming obvious in some parts of the range (Fig. 10). This neurone, for example, responded with a single spike at the beginning of each 20° ($200°s^{-1}$) movement except the 100-120° step (Insets in Fig. 10). It also fired at the end of 2 extension and 4 flexion steps ($P^{20}?A^{+-}$). The often high frequencies of tonic discharge made determination of acceleration

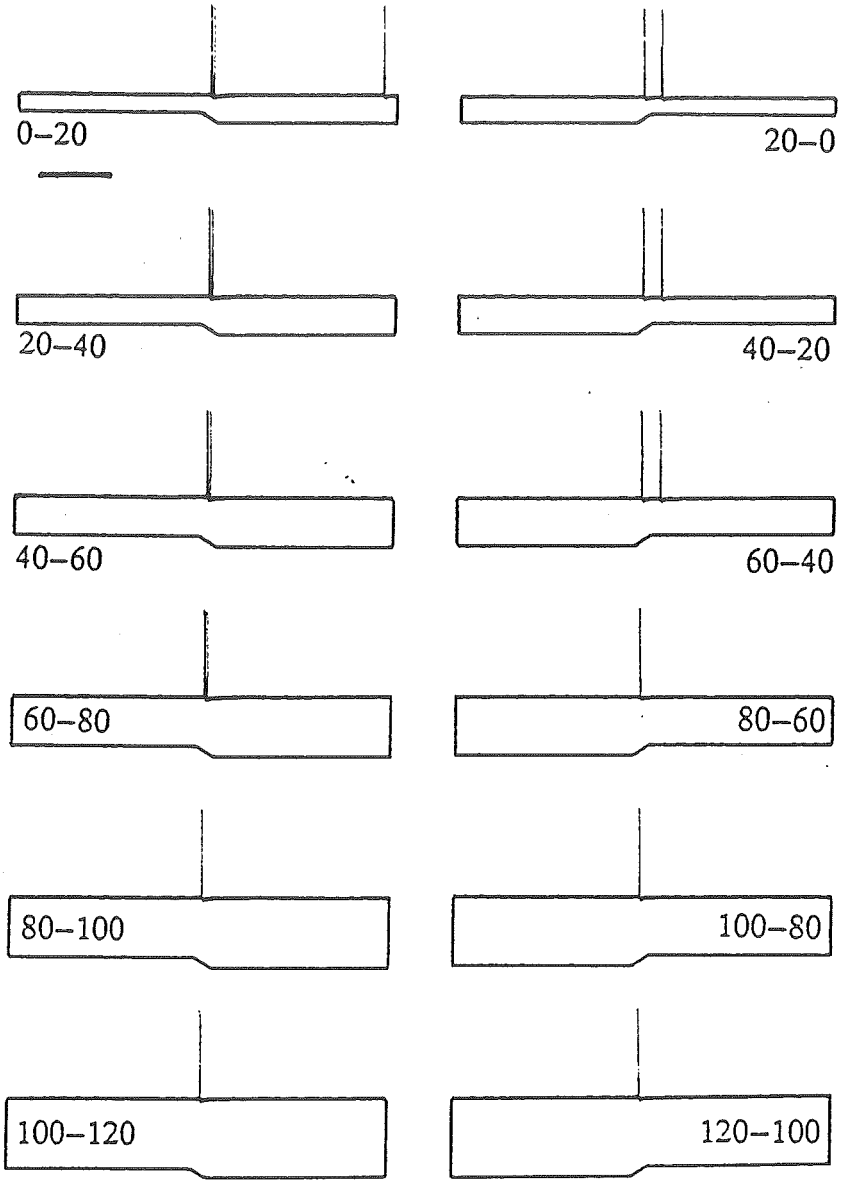


Figure 6. Acceleration receptor which fired at the start and ends of movements (?A^{+-} response). Each segment is taken from one continuous record (read down columns). Time scale: 0.1 sec. Spikes are 50 mV.

sensitivity difficult, so I do not feel justified in subdividing this 'position-and-acceleration' class on the basis of their acceleration components. Maximum tonic firing occurred at 0° (7 neurones), 20° (3), 40° (2), or 60° (3).

Most neurones which fired tonically had considerable hysteresis in their responses (Fig. 9b, arrows). The firing frequency at a set angle often differed by up to 10 Hz depending on whether the position was approached by extension or flexion (Fig. 8a-d). Movements of tracheae near the FCO sometimes affected the apodeme, causing variation in the tonic discharge rate at any given angle.

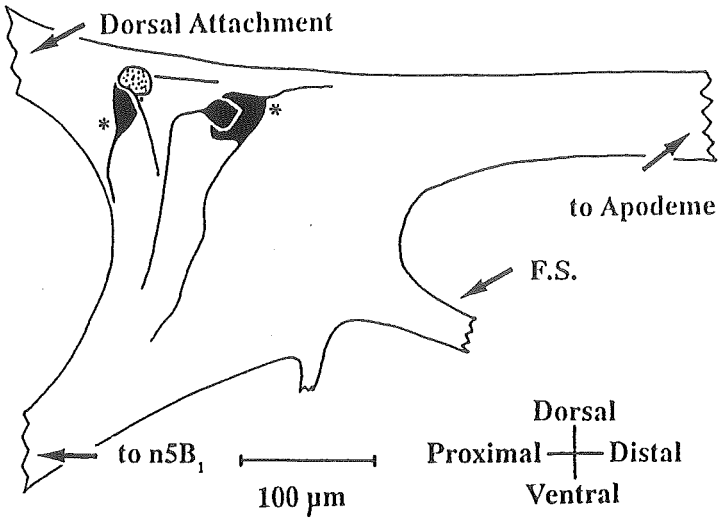


Figure 7. Position-and-acceleration sensitive neurones in the mtFCO. One stain was very light (*stippled soma*) and may not be accurate. P^{60}?A neurones are indicated by asterisks.

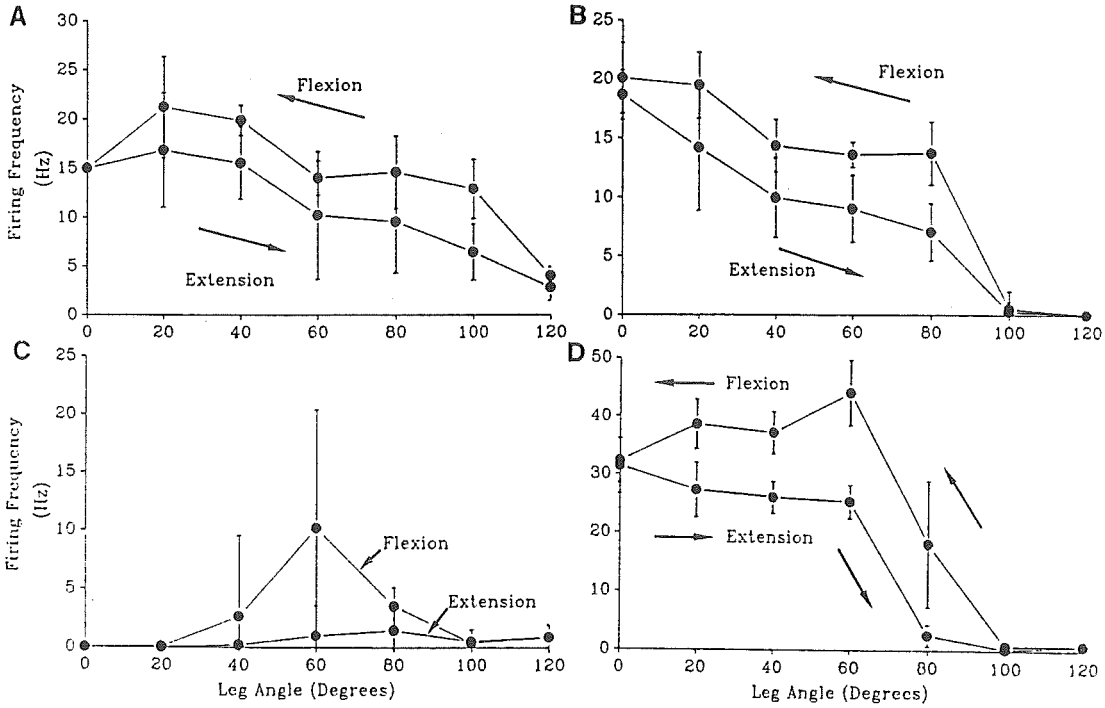


Figure 8 A-D. Tonic responses of the 4 position-and-acceleration sensitive neurones. Values are means and 99% confidence intervals: A,C: n = 3; B,D: n = 4. Considerable hysteresis is evident in all the responses. (A) A P^0?A neurone with gradual change in firing frequency. The low mean and large confidence interval (off axis) at 0° is caused by a low firing rate during one test at this angle. (B) A P^0?A neurone with a sudden change of firing frequency between 80-100°. (C) A P^{60}?A neurone with a gradual change in firing frequency. (D) A P^{60}?A neurone with a sudden change in firing frequency between 60-100°.

Position-and-velocity receptors

Only 3 of the possible combinations of position and velocity response were recorded (P^0V^+, P^0V^-, $P^{120}V^-$) (Table 1, Fig. 11a-d). P^0V^+ units were the most common receptor type encountered in this study. These neurones formed 2

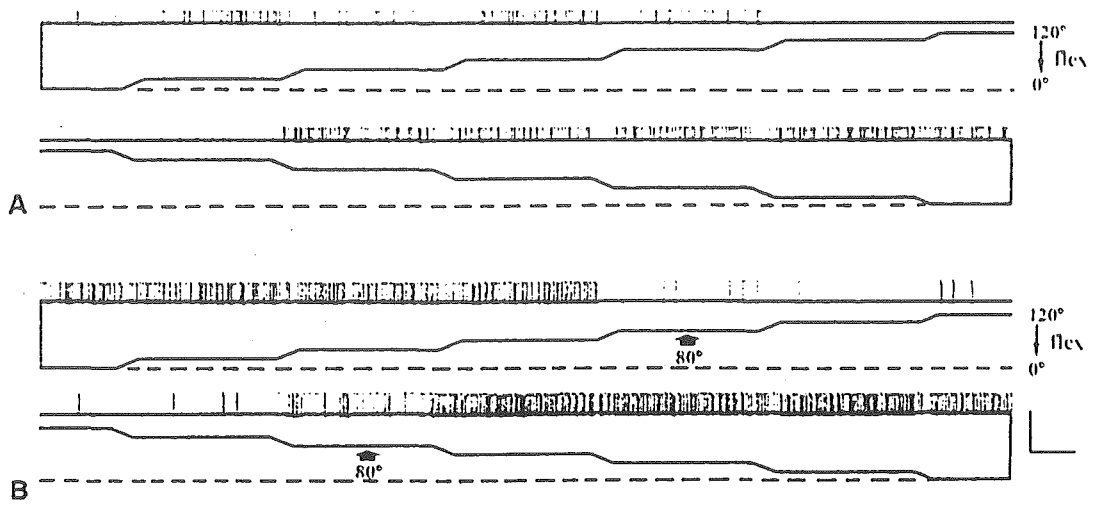


Figure 9 A,B. Response of position-and-acceleration sensitive neurones to 20° arcs at a velocity of $40°s^{-1}$. (A) A P^0?A neurone (cf. Fig. 8b) which is clearly inhibited during movements in either direction. (B) A P^{60}?A neurone (cf. Fig 8d) illustrating hysteresis in firing frequency caused by the history of leg movement (e.g., approaching a set angle (80° - arrows) from either a more flexed or more extended position. Scale: vertical; A = 62 mV, B = 44 mV: horizontal; A,B = 1 s.

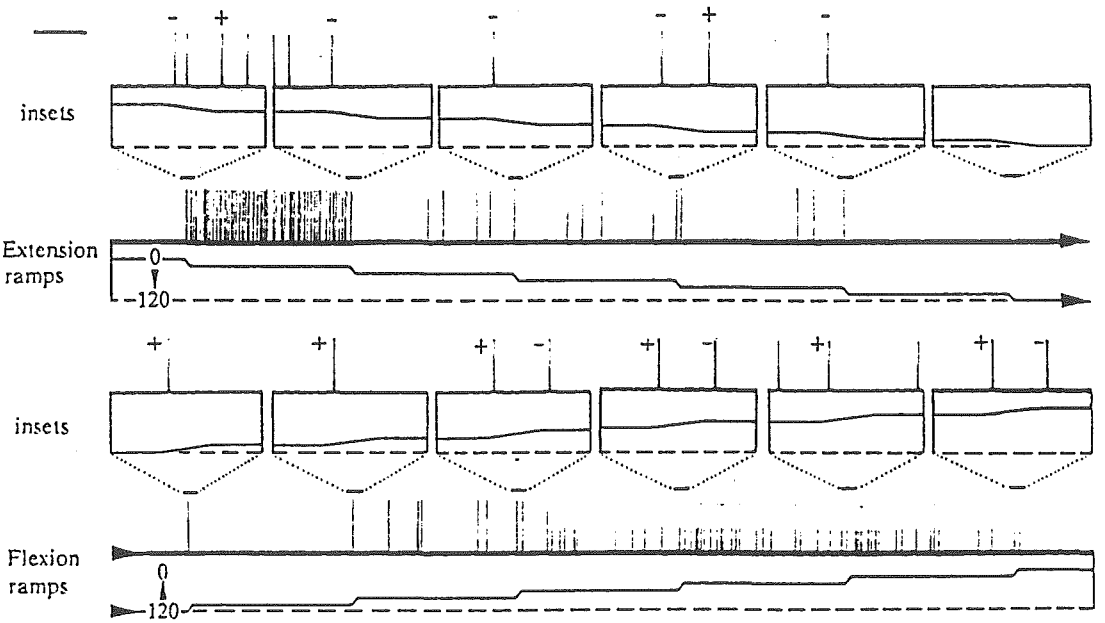


Figure 10. Position-and-acceleration receptor. Figure consists of a continuous record (extension ramps, flexion ramps), and insets of each phasic step (indicated by horisontal bars and dotted lines). The sign of acceleration causing phasic spikes is indicated by a plus or minus sign. Each step is a 20° movement. Time scale = 1 sec (0.1 sec for insets). Spikes are 35 mV.

clusters: one, containing 12 neurones, extended from near the dorsal attachment point distally for the length of the main body of the chordotonal organ (Fig. 11a); the other cluster (5 neurones) was in the centre of the chordotonal organ (Fig. 11b). The responses of the neurones comprising these two clusters differed. Those in the central group only fired tonically when the leg angle was between

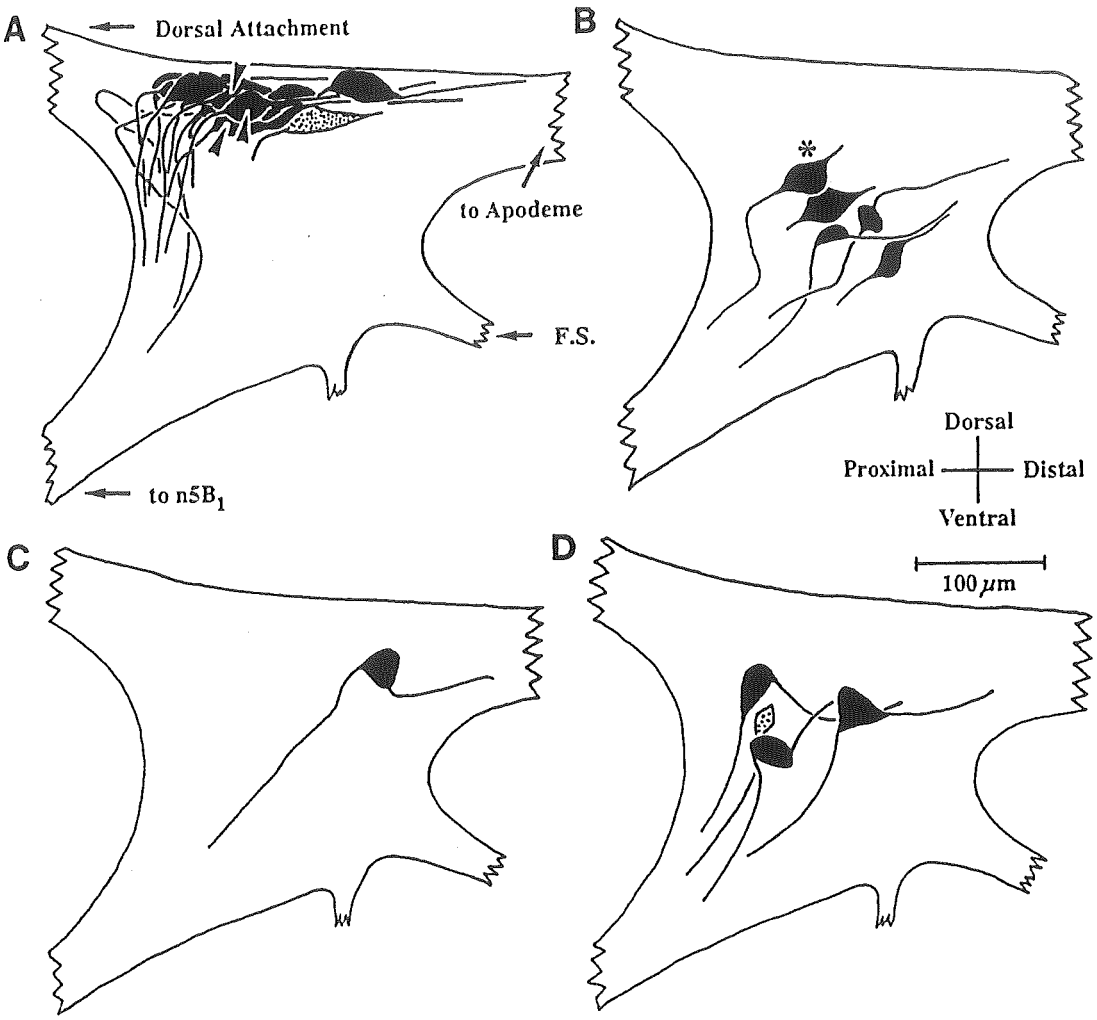


Figure 11 A-D. Locations of position-and-velocity receptors. (A) P^0V^+ neurones in this dorsal cluster responded to movements in a wide range of arcs. Three neurones (*arrowheads*) had maximum firing frequencies at 20°, and did not fire at 0°. The stippled cell was poorly stained, and may not be accurately represented. (B) P^0V^+ neurones in this central group responded tonically only between 0-20°, with 1 exception (*asterisk*). This cell fired tonically between 20-100°, with a maximum firing frequency at 40°. It did not fire at 0°. (C) The single P^0V^- neurone stained in this study was one of the most distal cells recorded. (D) $P^{120}V^-$ receptors were located in this central group. The stippled cell was poorly filled, and may not be accurately represented.

20-0° (Fig. 12a), while those in the dorsal group fired tonically over a wide range of leg angles (with maximal frequencies occurring near 0°) (Fig. 12b). The tonic firing rate of three cells in the dorsal group (arrowheads in Fig. 11a) increased as the leg flexed from 120° towards 0°, but reached a peak at 20°, and did not fire tonically when the leg was fully flexed. One cell in the central group (* in Fig. 11b) also differed from the general response pattern: it fired tonically at leg angles between 100 and 20°, with a peak firing frequency at 40°. It ceased firing when the leg reached 0°.

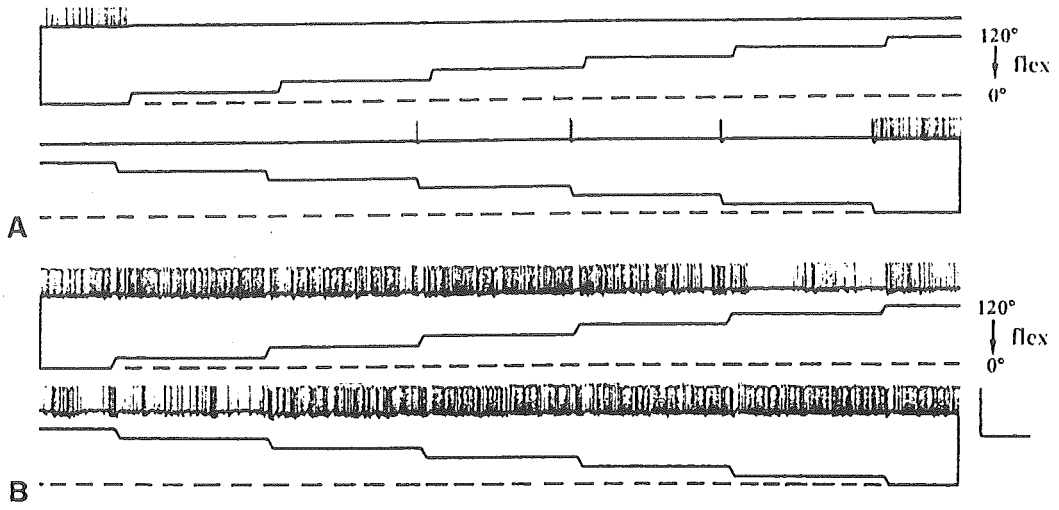


Figure 12 A,B. Responses of position-and-velocity receptors. **(A)** P^0V^+ Cells in the central group fired tonically only between 0-20°, and responded to flexions with a burst of spikes. **(B)** P^0V^+ neurones in the dorsal group fired tonically over a wide range of leg angles. Extensions caused inhibition of firing followed by a few rapid spikes before the tonic rate returned to a baseline level, while flexions phasically elevated the firing frequency, and were followed by a temporary reduction in tonic firing. *Scale*: vertical; A = 33 mV, B = 13 mV: horizontal; A,B = 1 s.

P^0V^+ neurones which responded tonically over a wide range of leg angles (i.e., those in the dorsal group) responded to flexions of the leg with bursts of spikes at a higher frequency than the tonic discharge. Movements anywhere in the leg's arc caused a phasic response. Extensions partially or completely inhibited tonic activity for the duration of the movement. After phasic bursts of spikes, the tonic discharge usually stopped for 0-220 ms (e.g., Fig. 12b). Conversely, the tonic discharge frequency was sometimes elevated for 0-400 ms following inhibition caused by leg extension.

The inhibition following phasic bursts of spikes is examined in more detail in Fig. 13. The responses of 10 P^0V^+ neurones to leg movements from 0° to 120° and back to 0° were standardised (as a percentage of the maximum tonic firing rate) and pooled. Following the stimulus the tonic firing frequency is clearly depressed for 100-300 ms. Only rarely did P^0V^+ neurones spike within 100 ms of such a stimulus.

The phasic response of P^0V^+ neurones in the central group was generally restricted to movements close to 0°. As velocity increased, movements closer to 120° also began to elicit phasic responses.

During the central projection study 5 neurones which had mid-position tonic maxima, and responded to the velocity of flexion were recorded ($P^{mid}V^+$ response). These included 1 neurone with a tonic maximum at 100°.

Only 1 neurone had a clear-cut P^0V^- response. This was one of the most

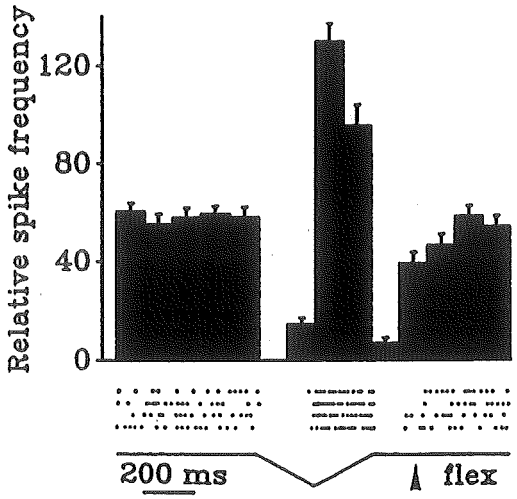


Figure 13. Post-excitatory inhibition in P^0V^+ neurones. The firing frequencies (relative to the maximum tonic rate) of 10 P^0V^+ neurones are plotted in the upper histogram (each bin is 100 ms wide). The bottom line indicates apodeme movement (0-120-0° at 600°s^{-1}): an upwards deflection (*arrowhead*) = flexion. The raster-type display represents the firing of one neurone during four consecutive stimuli: each dot represents one action potential. Following leg flexion, the neurones are clearly inhibited for up to 300 ms.

distally filled somata: it was located dorsally, near the point where the main body of the chordotonal organ narrows into the apodeme (Fig. 11c). This neurone fired tonically only at 0-20°, but responded phasically to extensions anywhere in the leg's arc of movement.

The 4 neurones which had a $P^{120}V^-$ response were located in a group close to, but slightly proximal from the centre of the FCO (Fig. 11d). These neurones only fired tonically between 80° and 120°, but responded phasically to movements between 40° and 120°. Higher velocities caused spikes during movements closer to 0°. There was no evidence for post excitatory inhibition.

Velocity-and-acceleration receptors

All of the 8 neurones which responded to the velocity of extensions and to accelerations ($V^-?A^-$, Table 1, Fig. 14) were located ventrally, close to the insertion point of the flexor strand. At high velocities (1000-400°s^{-1}, Fig. 15) each extension elicited a burst of spikes, and the end of most flexions elicited a single spike. At lower velocities (200°s^{-1}, Fig. 15) extensions near to 120° elicited only single spikes at the onset of each ramp (i.e., an $?A^-$ type response) while extensions near to 0° continued to elicit bursts. The end of flexions still evoked single spikes, but these occasionally failed (usually close to 0°). At yet slower velocities (67-40°s^{-1}, Fig. 15) $V^-?A^-$ neurones responded only to the onset of extensions (?A- response).

During the central projection study, 2 neurones were recorded which responded to the velocity of flexion and to positive accelerations ($V^+?A^+$: Fig. 16a,b). One of these neurones fired a single spike at the end of the first extension (0-20°: A^+ response), and responded to flexions between 40 and 0° with one

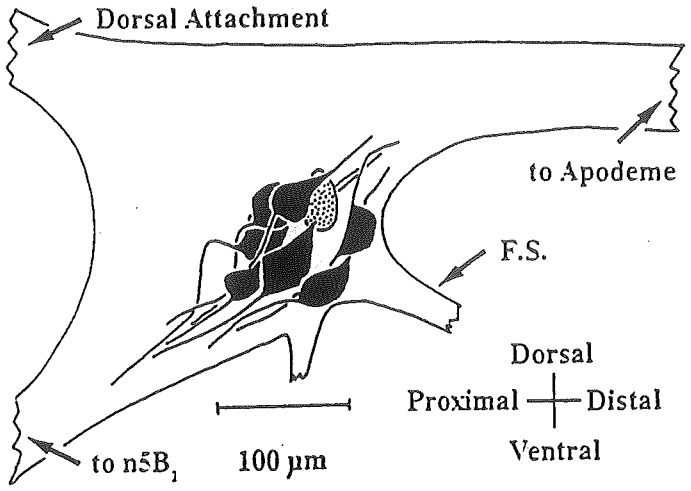


Figure 14. Locations of velocity-and-acceleration receptors ($V^-?A^-$). The stippled cell was poorly filled, and may not be accurately represented.

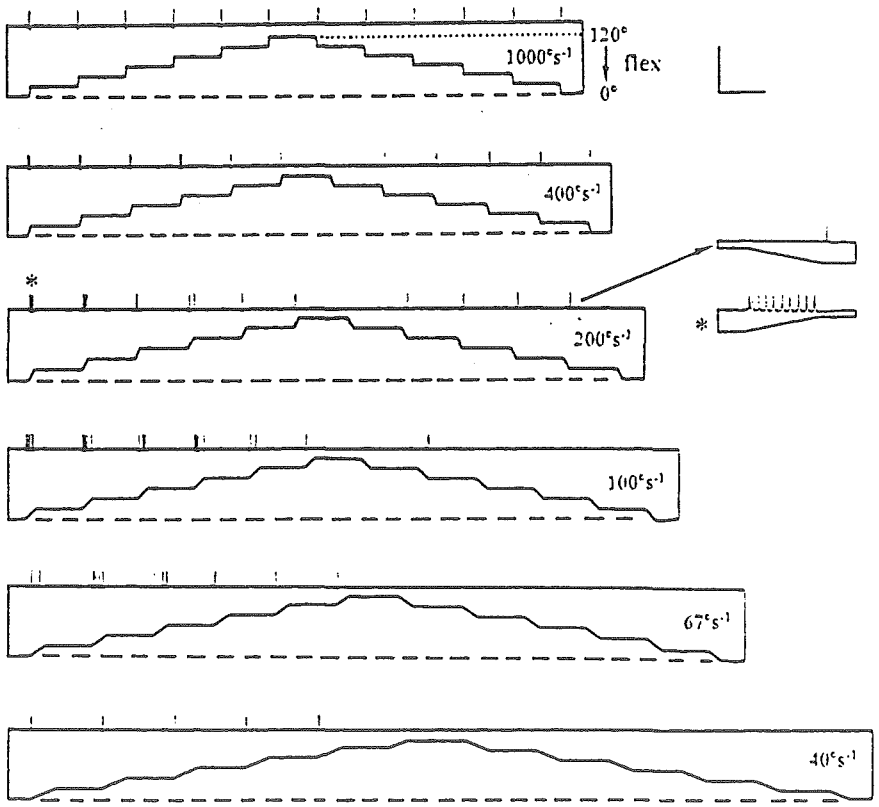


Figure 15. Responses of a $V^-?A^-$ receptor to movements of different velocities. The insets show at a faster sweep rate the timing of the spikes in relation to extension (*asterisk*) and flexion (*arrow*) movements. *Scale:* vertical = 35 mV: horizontal = 1 s (insets = 67 ms).

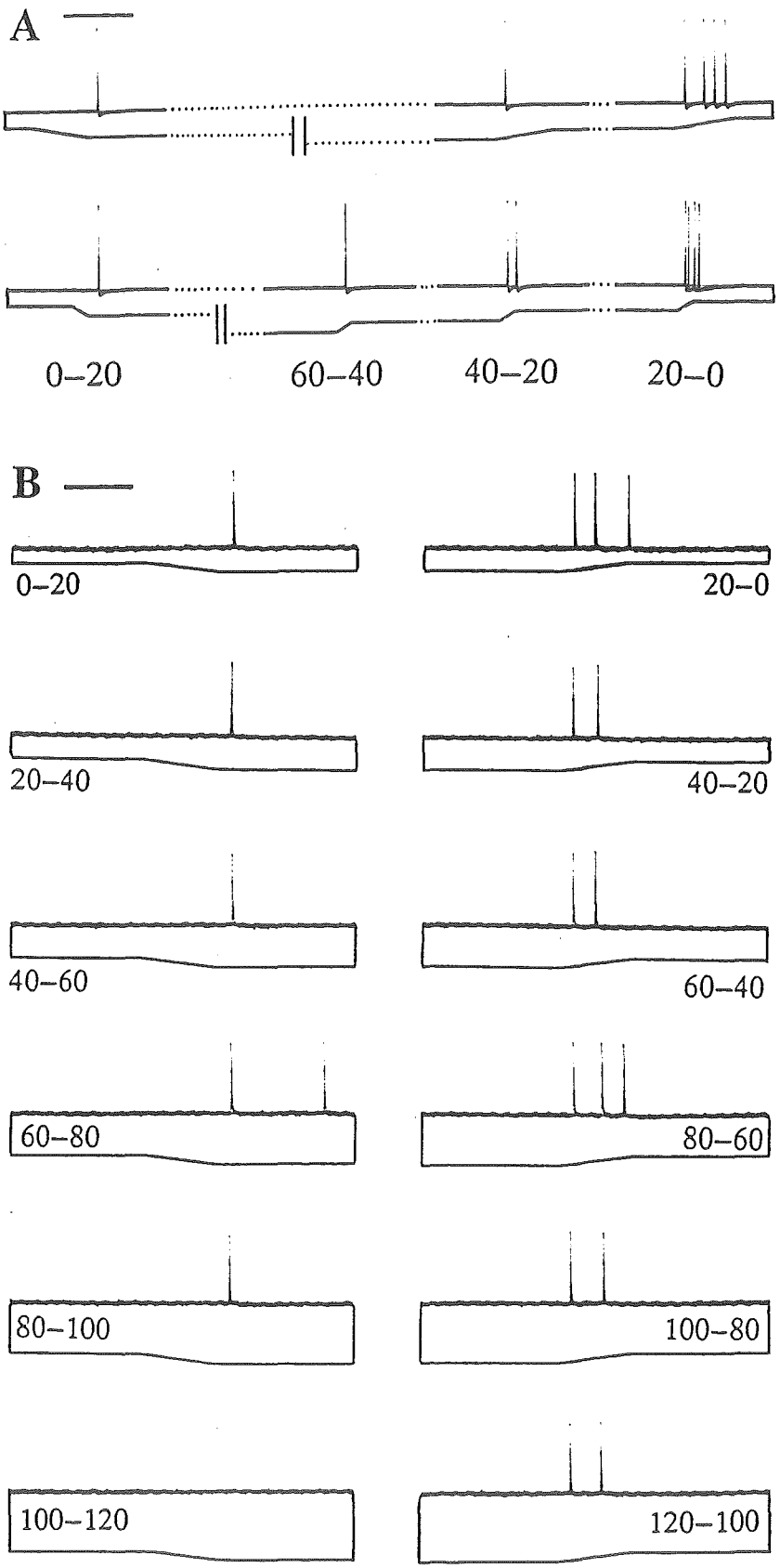


Figure 16 A,B. Velocity-and-acceleration receptors ($V^+?A^+$). The neurone in A only fired near 0°, while that in B fired at all angles. Time scale: 0.1 sec. Spikes in A are 55 mV, those in B are 20 mV.

or more spikes (including probable A^+ spikes) (Fig. 16a upper). At a higher velocity of leg movement the neurone also fired a single spike at the start of the 60-40° ramp (Fig. 16a lower).

The neurone represented in Fig. 16b fired a single spike at the end of each 20° (200°s-1) extension except the last (A^+ response), and responded to flexions at the same velocity with 2 or 3 spikes.

Position-velocity-and-acceleration receptors

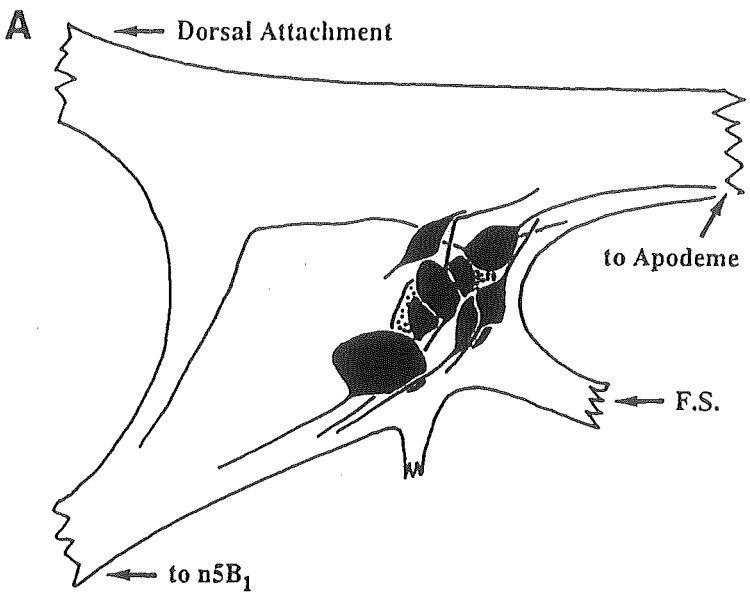
The most complex response types encountered in this study were $P^{(20\text{-}80)}V^-?A^-$, $P^{120}V^-?A^-$, $P^{(0\text{-}20)}V^+?A^+$, and $P^{(20}V^+)?A^-$. The 10 somata of the first type were located ventrally, at the point where the flexor strand inserts onto the chordotonal organ (Fig. 17a). These neurones had a tonic discharge across most or all of the leg's arc of movement, with a maximum firing rate between 20° and 80°. Extension movements anywhere between 0-120° elicited a burst of spikes lasting for the duration of the movement, while the end of flexions elicited a single spike (Fig. 18). Two neurones (with tonic maxima at 40° and 60° respectively) did not have a tonic discharge at 0°.

The only $P^{120}V^-?A^-$ receptor encountered was located in the centre of the FCO (Fig. 17b). This neurone fired tonically between 60-120°. Extensions anywhere between 0-120° elicited a burst of spikes, while the end of flexions elicited a single spike. At $40°s^{-1}$ extensions near 120° elicited only a single spike at the onset of each movement.

$P^{(0\text{-}20)}V^+?A^+$ and $P^{(20)}V^+?A^-$ neurones (Fig. 19a,b respectively) were recorded during the central projection study. The tonic maxima for individual neurones occurred at either 0° (4 neurones), 20° (2), 40° (1), or 20-60° (1). It is interesting to note that some spikes in Fig. 19a fall within the neurone's partial refractory period and are therefore reduced in height, while spikes at similar frequencies in Fig. 19b are not affected to such an extent.

DISCUSSION

Stretching or relaxing the chordotonal organ's apodeme excited neurones situated throughout the mtFCO. Interestingly, no neurones from the recently described distal cluster (Matheson and Field 1990) were successfully filled. These distal neurones are all small (somata diameters < 25 um, axon diameters < 2.6 um) and therefore probably difficult to impale with microelectrodes. In addition, even if stable penetrations were achieved, narrow axon diameters would restrict



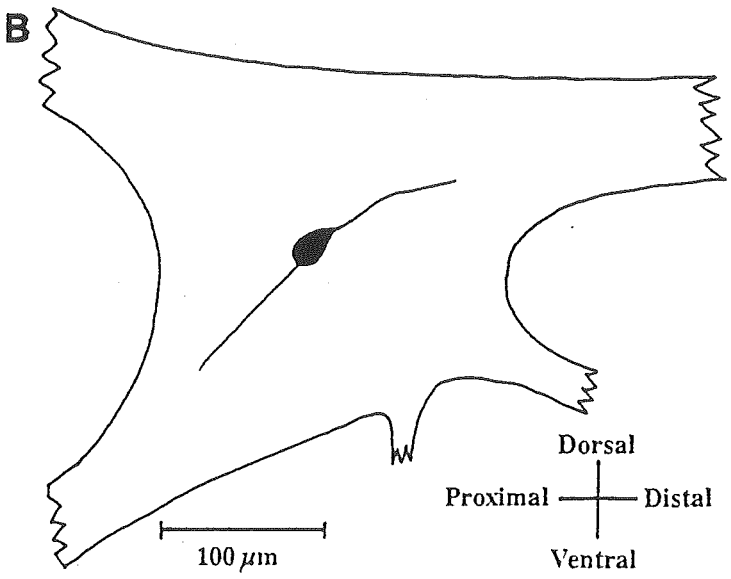


Figure 17 A,B. Locations of position-velocity-and-acceleration receptors. (A) $P^{20\text{-}80}V^{-}?A^{-}$ receptors were all located in this ventral group. The axon of 1 cell headed dorsally for a short while before turning towards n5B1. Two stippled cells were poorly filled, and may not be accurately represented. (B) The only $P^{120}V^{-}?A^{-}$ cell filled was located in the centre of the mtFCO.

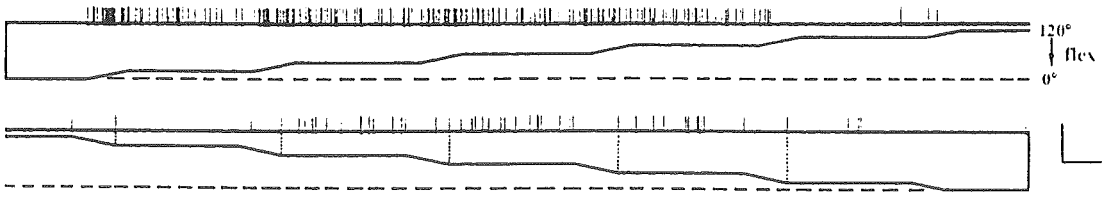


Figure 18. Response of a $P^{20\text{-}80}V^{-}?A^{-}$ neurone to $20°s^{-1}$ movements. Extensions elicit bursts of spikes at a frequency above that of the tonic discharge, while flexions reduce the tonic rate. The end of most flexions elicits a single spike. There is some evidence for a weak $?A^{+}$ response also, because the onset of some flexions elicits a spike. *Scale*: vertical = 50 mV; horizontal = 1 s.

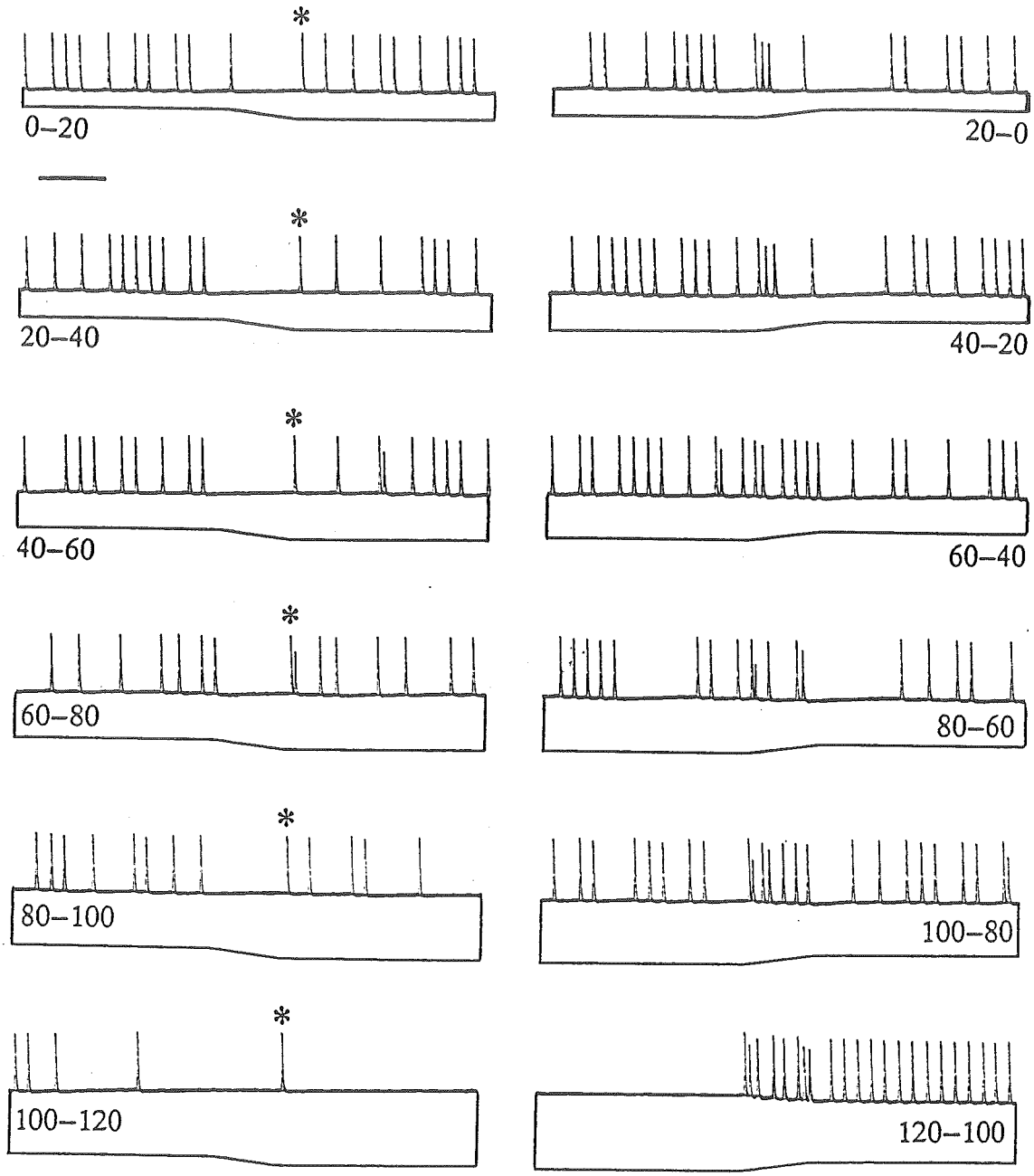


Figure 19 A,B. Position-velocity-and-acceleration receptors. The neurone in A has weak position and flexion-velocity responses, and fires a single spike at the end of extension movements ($P^{mid}V^{+}?A^{+}$). The neurone in B has a better defined response ($P^{20}V^{+}?A^{-}$). Note that these short segments of the complete record do not give a realistic indication of the tonic firing at each angle. Time scale: 0.1 sec. Spikes are 20 mV in A, 50 mV in B.

dye movement, resulting in unfilled somata. This may explain why a number of recorded cells failed to stain successfully. It is therefore not known what types of response the neurones in this substantial group of 40-50 distal cells have, or even if they respond to apodeme movements. Burns (1974) suggested that the small

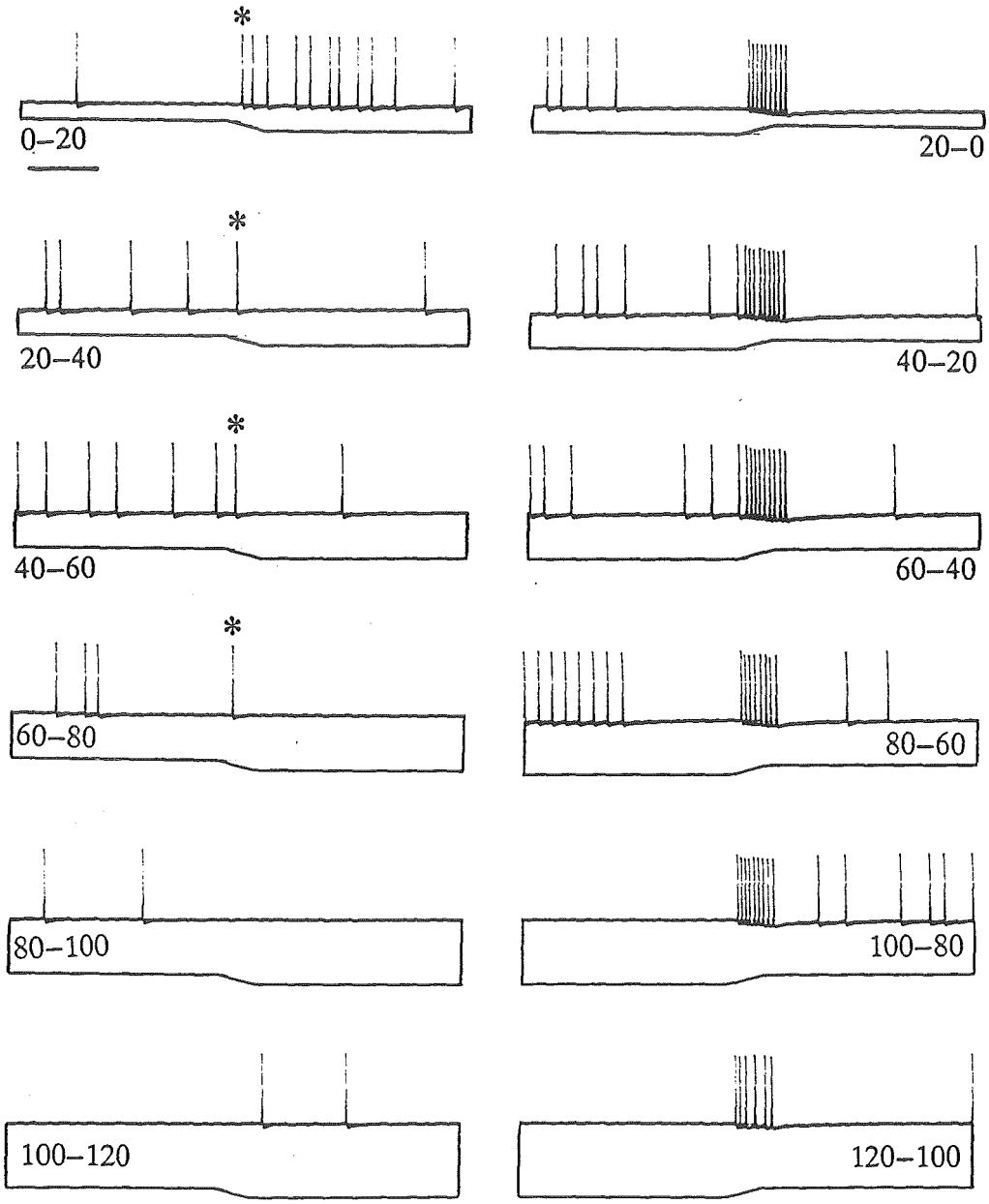


Figure 19 B. Caption on previous page.

neurones in the proximal scoloparium of the locust pro- and mesothoracic FCO's may be mostly position sensitive. If this was the case in the mtFCO distal cluster (the metathoracic homologue of the prFCO and msFCO proximal scoloparia (Matheson and Field 1990)) then it would explain the relative lack of pure position receptors noted in my study, and in that of Zill (1985a). Field and Pflüger (1989) recorded from the msFCO proximal scoloparium using hook

electrodes, and found that it contained both phasic and tonic (position sensitive) units. The msFCO proximal scoloparium does not seem to play any part in resistance reflexes, but may be important in the detection of vibrations (Field and Pflüger 1989). Recording intracellularly from the somata of mtFCO distal cluster neurones may be one way to determine their function, but membrane damage by the microelectrode to cells this size could pose considerable problems. The fine nerve in which their axons travel prior to joining the cuticular nerve (Matheson and Field 1990) is too short to allow a hook electrode to be used effectively.

FCO neurone response types

Twenty one major response classes were established (Table 1), some of which could be further divided into 2 or more subgroups by slight differences in their responses (a total of 24 distinguishable response types). Eleven response types found in this study were also reported from *Cuniculina* (V^+, V^-, $?A^+$, $?A^-$, $?A^{+-}$, P^0V^+, $P^{mid}V^+$, P^0V^-, $P^{mid}V^-$, $P^{120}V^-$, and $V^-?A^-$) (Hofmann and Koch 1985; Hofmann et al. 1985). It is not possible to compare in detail the relative numbers of each response type found in the two species because the apodeme movements used in my experiments are not expected to stimulate all the neurones within the locust FCO. General comparisons, however, are still meaningful.

Hofmann et al. (1985) did not note acceleration components in the responses of neurones classified as position receptors in the stick insect FCO. Zill (1985a) stated that all tonic neurones in the locust mtFCO had a phasic component, but it is not clear if this was in response to velocity or acceleration.

Hofmann et al. (1985) recorded from 3 P^{120} neurones, but my present work has revealed none of this type (or $P^{120}?A$ units) in the locust: the most-similar ones had tonic maxima at 60° ($P^{60}?A$). It is possible that $P^{120}?A$ receptors exist, but are stimulated by the flexor strand, rather than by the apodeme. All tonic neurones with mid-position sensitivity recorded by Hofmann et al. (1985) had a velocity-sensitive component. These neurones are also present in the locust, but are classified here as $P^{60}V^+$ or $P^{60}V^-$, rather than with pure position receptors. Some P?A type neurones in the locust have mid-position tonic maxima.

Position receptors are reported to have small somata and axons in both crustaceans and insects (Wiersma and Boettiger 1959; Hofmann et al. 1985; Zill 1985a), but this was not apparent in my study, probably because my technique (intracellular penetration of axons) biased the sample of neurones towards those with larger axons. Some position sensitive neurones recorded in my study re-

sponded to changes in leg angle with only small alterations of firing frequency over most of their response range, but with large changes in firing frequency between 60-100°. These angles (which correspond closely to those used during normal walking (Burns 1974)) are therefore coded more precisely than angles in any other part of the leg's travel.

In their description of stick insect walking, Weiland and Koch (1987) make the assumption that some position sensitive neurones signal when the leg reaches a particular "critical angle" close to the end of its movement arc. This signal initiates 'end point behaviour' (i.e., the excitation of muscles opposing the ongoing movement). The velocity sensitive neurones which respond near the extremes of leg movement are ideally suited to initiate end point behaviour since they are particularly sensitive at the appropriate angles.

Neither Zill (1985a) nor I recorded from any V^{+-} receptors, yet Hofmann et al. (1985) encountered these relatively often. This difference between locust and stick insect thus appears to be real. Bidirectional velocity receptors have also been found in an abdominal chordotonal organ of *Carausius* (Orchard 1975) and in crustacea (Taylor 1975).

In my study some velocity receptors had a low frequency erratic discharge, independent of leg angle, but this was always related to movements of tracheae close to the FCO. Similarly, erratic spikes were sometimes seen in recordings from acceleration receptors. These also were largely related to tracheal movements. I did not find any acceleration receptors with high frequency background discharges unrelated to leg angle as reported by Hofmann and Koch (1985). The tonic discharges of similar units in the locust were clearly related to leg angle (position-and-acceleration response).

Bässler et al. (1986) noted that in resting *Cuniculina*, accelerations at the start, but not at the end of relaxation movements caused depolarisation of the FETi motorneurone. Hofmann and Koch (1985) reported that most acceleration receptors responded more strongly when the sign of the acceleration was the same as that of the velocity (i.e., they fired at the start rather than the end of movements). The neurones that I have classified as ?AS follow this pattern of response, but the exact reason for this remains unclear because accelerations were not controlled.

My failure to note any $P^{120}V^{+}$ or $V^{+}?A^{-}$ receptors is in agreement with their relative scarcity in the stick insect, and probably does not indicate a real difference between the species. Two $?A^{+}$ receptors and 2 $V^{+}?A^{+}$ units (from the central projection study) were found in my investigations, but have not been

described from the stick insect. This apparent absence probably reflects the small sample sizes rather than a significant difference (I recorded 17 $V^-?A^-$ and 2 $V^+?A^+$ units, while Hofmann et al. (1985) recorded 5 $V^-?A^-$ and no $V^+?A^+$ units).

The apparent lack of PVA neurones in the stick insect (Hofmann and Koch 1985; Hofmann et al. 1985) has been used as evidence that different afferents converge onto single interneurones (Büschges 1989). VA neurones in the stick insect often had high levels of spontaneous activity, apparently unrelated to leg angle. This was not observed in my experiments: any 'tonic' firing was either clearly related to visible tracheal movements, or to leg angle. Hofmann and Koch (1985) do not clearly state what leg angles were used to test for position sensitivity of acceleration receptors, and do not show original records of any spontaneously active VA receptors. In light of my findings therefore, a further detailed analysis (covering all possible leg angles) of the nature and causes of the spontaneous firing seen in stick insect afferents would be illuminating. It is interesting that although I have recorded no $V^+?A^-$ neurones, one $P^{20}V+?A^-$ unit has been identified.

Most neurones had wide response ranges, extending from one or other extreme towards the central (60°) position (both tonic and phasic components). This is in keeping with the increase in overall FCO activity seen in extracellular records at extreme leg angles (Usherwood et al. 1968; Burns 1974; Zill 1985a). Some range fractionation occurred (see Chapter Three). Acceleration sensitive units often responded equally strongly over the entire range of leg angles, as did some velocity receptors when stimulated well above threshold. Tonic (position sensitive) neurones sometimes had peak firing frequencies away from the extremes. In particular, $P^{20\text{-}80}V^-?A^-$ units always did this, sometimes failing to fire at all at 0°. These findings contrast to those of Zill (1985a) who reported that the firing rate of all tonic units increased towards the extremes. In functional terms there are two points to consider: (1) The range of angles over which a neurone has its maximum firing frequency does not necessarily correspond to the range of angles over which it is most sensitive. For example, some position sensitive neurones recorded in this study are most sensitive at the end of their response range, where small movements cause large changes in firing frequency; (2) locusts do not use all the possible leg angles with equal frequency (e.g., Burns 1973). M. Coles (1988, unpubl.) has clearly shown that resting locusts have preferred leg angles. When animals are resting on a horizontal surface, the metathoracic leg is preferentially held at either 0-9°, or at 30-60°. Locusts on

vertical surfaces almost exclusively hold their metathoracic legs at 0-9°. These angles are monitored accurately by position sensitive neurones (my study).

Hysteresis in the responses of chordotonal organ neurones is well established (Usherwood et al. 1968; Burns 1974; Hofmann et al. 1985; Zill 1985a; Zill and Jepson-Innes 1988; this study) yet Burrows (1988) states that "the changed pattern of FCO afferent spikes is complete within 100 ms of the end of the stimulus". He then goes on to reason that "the effects in the spiking local interneurones here appear to outlast changes in the afferent signals...and must therefore have their origins in the local interactions in the metathoracic ganglion". While large phasic units may fire only during movements, smaller tonic ones have modified firing rates for 200 ms to 1 min following a step displacement and return (Zill 1985a, this study). These units seem to have been overlooked by Burrows (1988), perhaps because their spikes were obscured in his extracellular records. The inhibition of position-and-velocity sensitive neurones recorded here (Fig. 13), and noted by Zill (1985a) probably functions to counter muscle catch tension (Zill and Jepson-Innes 1988). Post-excitatory inhibition of FCO afferents has not been explicitly reported from the stick insect, although some of the original recordings in Hofmann et al.'s (1985) paper suggest that it does occur in *Cuniculina*. Other figures in the same paper, however, do not show this (Compare their Figs 5 and 9b with their Figs 3, 4, 6, 7, and 10).

Weiland and Koch (1987) have shown that velocity is controlled during voluntary leg movements in *Carausius*. With a few exceptions V^+ units recorded in my study responded more strongly in ranges of leg movement close to 0° (+), while V^- units responded more strongly as the leg approached 120° (-). If these neurones are involved in velocity control feedback pathways (as is likely) then the implication is that velocities are regulated more accurately as the leg approaches the limits of its movement (although this was not tested by Weiland and Koch).

It is perhaps significant that some V^+ and P^0V^+ receptors only became active when the leg was between 0-20°. These neurones could be important during jumping or kicking, when the leg must be held fully flexed in order to develop power (Heitler and Burrows 1977). The opposite situation (V^- receptors with sensitivities restricted to near 120°) was not encountered. Some neurones responded to stretch of the apodeme (flexion), while others clearly responded (by spiking) to relaxation of the apodeme (extension). This confirms Zill's (1985a) suspicion that "...[some] receptors may respond to relaxation rather than stretch...". In my experiments the flexor strand was held stationary at 60°, so it could not have contributed to this response.

FCO neurone somata locations

Zill (1985a) recorded from somata in known regions of the locust mtFCO, but did not provide a map of cell sizes and positions, relying instead on verbal descriptions. He reports that all extension sensitive phasic neurones and tonic neurones active between 80-170° were found in a ventral group, with dendrites at an angle to the apodeme, while flexion sensitive phasic neurones, and tonic neurones active between 0-80° were located dorsally, with their dendrites parallel to the apodeme. Neurones in the crab propodus-dactylus (PD) organ are also spatially ordered with respect to their responses (Wiersma and Boettiger 1959; Hartman and Boettiger 1967).

The maps of cells in the present study show that FCO neurones exhibiting similar responses have somata in comparable locations, and dendrites with similar orientations. The pattern of distribution, however, is more complicated than previously suggested. Zill's (1985a) Fig. 3b shows the scolopale orientations in one FCO (as determined from a sectioned preparation) being unequivocally divided into 2 orientations: either parallel, or at 30-35° to the receptor apodeme. Field and Matheson (unpublished) note that FCOs fixed and dehydrated in situ, dissected out of the leg and then viewed under interference contrast optics, often clearly show a range of scolopidial orientations, not a clear-cut division into 2 populations. This observation is supported by the diversity of angles of dendrites recorded in Figs 2a-c, 5a-c, 7, 11a-d, 13 and 17a,b.

In my study, neurones with somata located dorsally included all P^0, P^{60}, $?A^+$, $?A^-$, and ?AS receptors, and some V^+, V^-, and P^0V^+ units. Neurones with ventrally located somata included all $P^{20\text{-}80}V^-$, $P^{120}V^-$, $P^0V^-?A^-$, and $V^-?A^-$ units, and some V^+, V^-, and P^0V^+ receptors. It appears that division of the mtFCO into two groups of neurones ('dorsal' and 'ventral') on the basis of extension or flexion sensitivity is artificial and inaccurate. It is interesting to note that the ventrally located P^0V^+ and V^+ neurones, and the most ventral V^- unit all had narrow response ranges restricted to angles close to 0°.

Movement of the apodeme alone (i.e., with the flexor strand fixed at 60°) is sufficient to excite both flexion and extension sensitive neurones throughout the mtFCO. Future investigations should deal in detail with the distribution and responses of neurones excited by flexor strand movements, and with possible interactions caused by simultaneous movement of both attachments.

CHAPTER THREE

Range fractionation in the locust metathoracic femoral chordotonal organ

SUMMARY

Insect femoral chordotonal organs are internal stretch receptors which monitor the position and movement of the femur-tibia joint of the leg. Intracellular recordings from neurones in the metathoracic femoral chordotonal organ (mtFCO) of *Locusta migratoria* have been used to investigate range fractionation and hysteresis of tonic and phasic responses to tibial movements. Some neurones respond across the full range of femur-tibia angles, while others have restricted response ranges, and could act as labelled lines. Neurones with maximal firing at mid-angles are described for the first time in the locust mtFCO. Problems associated with previous extracellular studies are discussed. Responses are discussed in terms of underlying structural constraints on signal transduction. The problems of developing a viscoelastic model of the mtFCO are outlined, and an alternative method is suggested.

INTRODUCTION

Often sensory systems must often be able to respond to stimuli which vary in intensity over several orders of magnitude. In addition, they must be able to represent any relevant stimulus with a certain degree of precision. Because of the limited range of firing frequencies available to single neurones, these two requirements are often compromised one against the other. For example, Type 3 receptors of the decapod PD organ which code for wide ranges of stimulus intensities are often quite insensitive (Mill and Lowe 1972). On the other hand, sensitive receptors often respond only to a restricted subset of possible stimuli (e.g., some *Decticus* primary auditory neurones (Kalmring et al. 1978)). Proprioceptors which monitor appendage joint angles face a further complicating factor: they must unambiguously represent a parameter of movement such as velocity or acceleration over a range of leg angles. These neurones' firing frequencies may therefore be influenced by both velocity and absolute leg angle.

By increasing the number of receptor units (neurones) within a sense organ, it is possible to overcome these problems. At least 2 scenarios are possible: (1) each unit responds to the full range of stimuli, but is relatively insensitive. The sensitivity of the receptor organ as a whole is increased by subsequent convergence of afferent information onto interneurones (Aidley 1978,

and see Fig. 5 in Hofmann et al. 1985); (2) individual units respond with great accuracy, but only within narrow ranges of stimulus intensity. Different neurones respond in discrete (or slightly overlapping) ranges, in such a way that the combined response of the organ codes for all stimuli (range fractionation) (Cohen 1963). If a neurone's response range is sufficiently narrow, then that neurone may function as a 'labelled line'. The movement units of the crustacean myochordotonal organ (MCO_2) function in this way. Their firing frequency codes for joint velocity, while the presence of firing indicates that the leg is within the response range (position information). Evoy and Cohen (1969) suggest that this type of neurone may be necessary in MCO_2 to allow fine control of movement. Many vertebrate and invertebrate sensory systems exhibit range fractionation. These include taste and temperature receptors in the cat tongue (Cohen et al 1955, Dodt & Zotterman 1952); arthropod limb proprioceptors (Burns 1974, Cohen 1963, Hofmann et al. 1985, Young 1970, Zill 1985a); insect auditory receptors (Kalmring et al. 1978, Rheinlaender 1975); insect mechanoreceptors (Shimozawa & Kanou 1984a,b); insect vibration receptors (Kühne 1982); equilibrium receptors in vertebrates (Lowenstein & Roberts 1950); and invertebrate statocysts (Cohen 1960).

The femoral chordotonal organs (FCOs) of insect legs are multineuronal sense organs which monitor movements of the tibia relative to the femur. They have a role in shaping leg movements (Hofmann et al. 1985, Usherwood et al. 1968, Zill 1985). FCO afferent neurones make direct connections with motor neurones, spiking and non-spiking local interneurones, and interganglionic interneurones (Burrows 1987, 1988, Burrows et al. 1988, Laurent 1988, Siegler 1984, Wilson 1981). Several authors have mentioned range fractionation in insect joint chordotonal organs (Burns 1974, Hofmann et al. 1985, Matheson 1990, Zill 1985a), but, in contrast to the locust auditory system (Kalmring et al. 1978, Rheinlaender 1975), there has not yet been a thorough study of this aspect of sensory function in joint chordotonal organs. Matheson (1990) outlined the types of receptor neurones in the mtFCO, while a second report (Matheson in prep: Chapter Four) describes the relationship between response type and central projection pattern. Field (in press) has recently described a possible mechanical contribution to range fractionation in the mtFCO, but this has not yet been correlated with detailed physiological studies. Hofmann et al. (1985) have discussed aspects of range fractionation in the stick insect FCO, giving examples of the main response types. This valuable information however, was not presented in a way that gave an appreciation of the overlap between response ranges; nor did

the few examples reveal much about the more subtle differences between neurones (because the examples tend to be either 'typical' of a class, or 'extreme').

The main aim of my present paper is to illustrate and discuss the response ranges, variation between units, and overlap of responses in the locust mtFCO. I have recorded intracellularly from individual locust metathoracic FCO (mtFCO) neurones while moving the FCO apodeme to mimic leg movements in the range 0-120° (encompassing the angles used by the locust during normal activities). I examine both tonic (position) and phasic (velocity) range fractionation. Because the acceleration of movements was not controlled I do not describe the range fractionation of putative acceleration receptors. The hysteresis of the tonic responses is also illustrated and discussed.

MATERIALS AND METHODS

Adult locusts (*Locusta migratoria*) from our laboratory culture were used for all experiments. The results are based on 189 successful recordings from 256 animals. These results are derived from experiments reported in Matheson (1990) and Matheson (in prep) (Chapters Two and Four).

The terminology, and the stimulating and recording techniques used have been fully described previously (Matheson 1990, in prep), and are only briefly mentioned here for clarity.

Animals were restrained ventral side up in plasticene. Movements of the mtFCO apodeme at controlled velocities mimicked tibial movements between 0 and 120°. The flexor strand was held static at 60°. Intracellular recordings were made from FCO axons either close to the metathoracic ganglion (n5: Matheson in prep), or in mid-femur ($n5B_1$: Matheson 1990). Drops of locust saline (pH 6.8) were added if required to prevent drying out of the preparation. Responses to stepwise ramp-and-hold stimuli at a range of velocities were recorded on tape for later filming and analysis. Position and velocity (but not acceleration) of the apodeme were controlled. Putative acceleration receptors have been discussed by Matheson (1990), and the term 'acceleration receptor' is used in the same way here to refer to these units.

RESULTS

Classification of receptors

The terminology used here to describe FCO neurone's response types follows that described in detail by Matheson (1990). If a neurone had a tonic discharge

while the femur was held at a constant angle, the neurone was deemed to be position sensitive (abbreviation: P). The angle at which the highest firing frequency occurs was added as a superscript (e.g., P^{20}). The abbreviation P^x is sometimes used to refer to a group of tonic neurones irrespective of their individual tonic maxima. Neurones which responded to leg movements in a velocity dependent way were given a 'V' code. The direction of movement that excited the neurone was added as a superscript 'plus' or 'minus' sign (+ = tibial flexion, - = tibial extension). If the neurone matched the criteria for acceleration sensitivity (see Matheson 1990), it was given a '?A' code. Again, the sign of acceleration that excited the neurone was added as a superscript 'plus' or 'minus'. Acceleration sensitive neurones which responded to both signs were classed as $?A^{+-}$. Another type of acceleration sensitive neurone fired in response to both signs of acceleration, but only at the start of movements. These were classed as '?AS' neurones (for "Acceleration at the Start"). When a neurone responded to more than one parameter of a movement the appropriate abbreviations were combined.

Range fractionation

Tonic responses

I present the tonic neurones in order of their position sensitivity, beginning with those that fire maximally at 0°, and progressing to those with highest firing frequencies at 120°. All tonically active neurones (including the various phasic-tonic response types) have been pooled in this section. The full response type of any particular illustrated neurone is given in the appropriate figure legend.

During recording in my experiments neurones were selected so that I had examples of as many response types as possible. For this reason it is not valid to compare the number of neurones having maxima at each angle. In fact, there were many more neurones with maxima at either 0 or 120° than with maximum firing rates at mid-angles.

The tonic values plotted for each neurone are averages taken from at least 3 (usually 4-8) complete 'staircase' (ramp-and-hold) stimulations (each with ramps at a different velocity). Error bars indicate ±1 SE, and are often hidden by the size of the symbols. In cases where hysteresis caused an ambiguity in determining the leg angle at which maximal firing occurred, the two curves were averaged.

Maximum at full flexion (0°). Neurones with tonic maxima at 0° included 4 which

were relatively insensitive to position between 0 and 80°, but which responded with a marked reduction in firing to more extended angles (Fig. 1A, and upper curve in Fig. 1D). These neurones all had relatively high firing frequencies at 0° (>25 Hz). Other neurones had a more linear response curve (Fig. 1B). It is likely that there is a continuum of response type, rather than separation into 2 distinct types. For example, the neurone represented in the upper curve in Fig. 1B has the steepest part of its response between 80 and 120°, yet does not have a plateau between 0 and 80°.

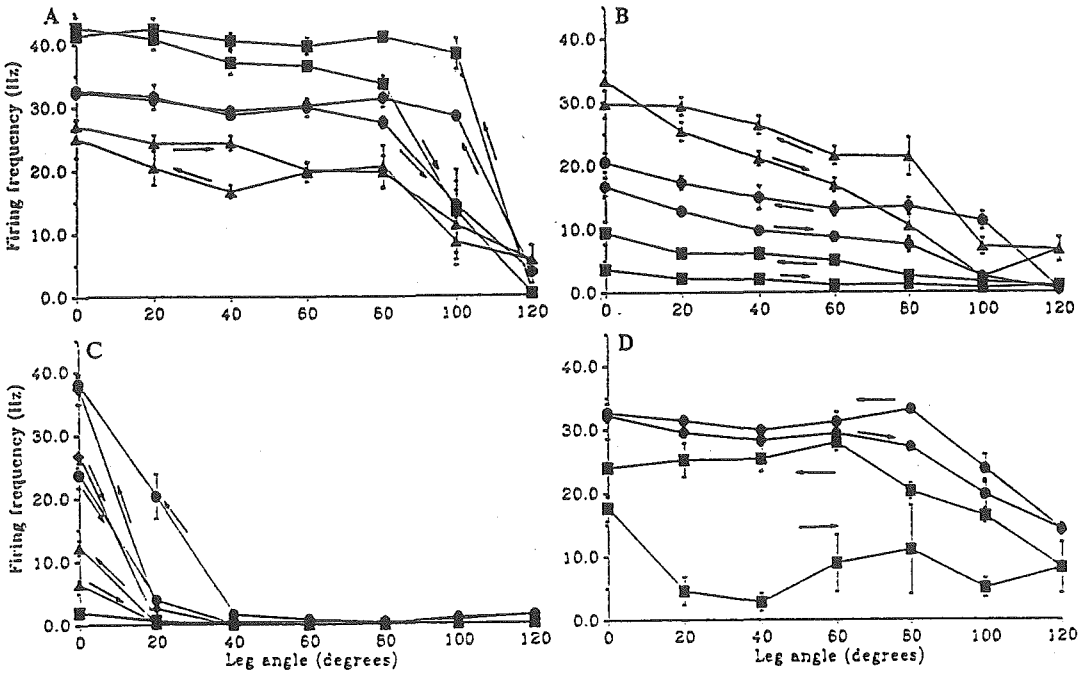


Figure 1 A-D. Tonic neurones with maximal firing at 0°. (A) Units with a sharp reduction in firing between 80 and 100°. *Circles, squares* = $P^0V^+?A^+$, *triangles* = P^0V^+. (B) Units with a gradual decrease in firing at extended angles. *Circles* = $P^0?A^+$, *squares, triangles* = P^0V^+. (C) Units with ranges restricted to near 0°. *Circles, diamonds* = P^0V^+, *squares* = $P^0?AS$, *triangles* = P^0V^+. (D) Comparison of hysteresis. *Circles* = P^0V^+, *squares* = P^0V^+.

Thirteen other neurones with tonic maxima at 0° had narrow response ranges restricted to angles between 0 and 40° (Fig. 1C). Generally these neurones had maximal firing rates of less than 25 Hz, but one (upper curve in Fig. 1C) reached 40 Hz at 0°. These neurones vary rarely fired tonically at angles greater than 40°.

Hysteresis in the response curves was evident in varying degrees for different neurones, as evidenced in Fig. 1A-C (and see Discussion). Fig. 1D compares the extent of hysteresis in 2 P^0 neurones with similar maximum tonic firing rates. The lower curve represents one of the most extreme cases recorded, with a

hysteresis in excess of 20 Hz at a leg angle of 40° (approx 80% of its maximum firing rate). This neurone was also quite sensitive to velocity of flexion (P^0V^+ response), but there was no evidence that the tonic firing rate following flexions was affected by the velocity of the preceding movement. Other, equally sensitive, P^xV neurones did not exhibit such a large hysteresis.

Maximum at 20°. Two of the 8 neurones with a tonic peak at 20° had maximum firing frequencies of 60-80 Hz (e.g., upper curves in Fig. 2A,B), although most maxima were at less than 40 Hz. Two types of curves were apparent (contrast Fig. 2A and B). The firing of some neurones falls off in a linear way for angles greater than 20° (Fig. 2A), while others (Fig. 2B) have a broad plateau between 20° and approximately 80°, with relatively steep drop-offs at either extreme of leg movement. Even neurones with low firing frequencies (e.g., lowest curves in Fig. 2A and B) show the same trends (Fig. 2 Inset: note vertical scale). Note that the hysteresis of the neurone shown in the upper curve of Fig. 2B reverses at a leg angle of 60°.

Maximum at 40°. Neurones with tonic maxima at 40° (n = 13) exhibited similar response patterns to those described for P^{20} neurones. Some (Fig. 3A upper curve) had a broad plateau from 0-60°, with a sharp decline at more extended angles, while others had a steady decrease between 40 and 120° (lower curve). In contrast to these types, most P^{40} neurones had quite low firing rates at both 0° and 120° (Fig. 3B-D). Some had ill-defined peaks (Fig. 3B), while others had more clear-cut responses (Fig. 3C, and especially Fig. 3D). Hysteresis was present to varying degrees, with some neurones having markedly different firing rates following ramps in either direction (upper curve in Fig. 3C), while others showed little difference (lower curves in Fig. 3C,D). Neurones of the former type often did not have a tonic response during holds following one direction of movement (lower curve in Fig. 3C). The hysteresis of some curves crossed over between 60 and 80° (Fig. 3D).

Maximum at 60°. The responses seen for other mid-position sensitive neurones were present in P^{60} neurones (i.e., plateau from 0-60° with sharp drop-off to 120° (Fig. 4A); broad peak of maximum firing (Fig. 4C); variable hysteresis (compare curves in Fig. 4C)). None of the 6 P^{60} neurones had response curves in which the hysteresis crossed over.

Some P^{60} neurones had quite narrow peaks of tonic firing (Fig. 4D).

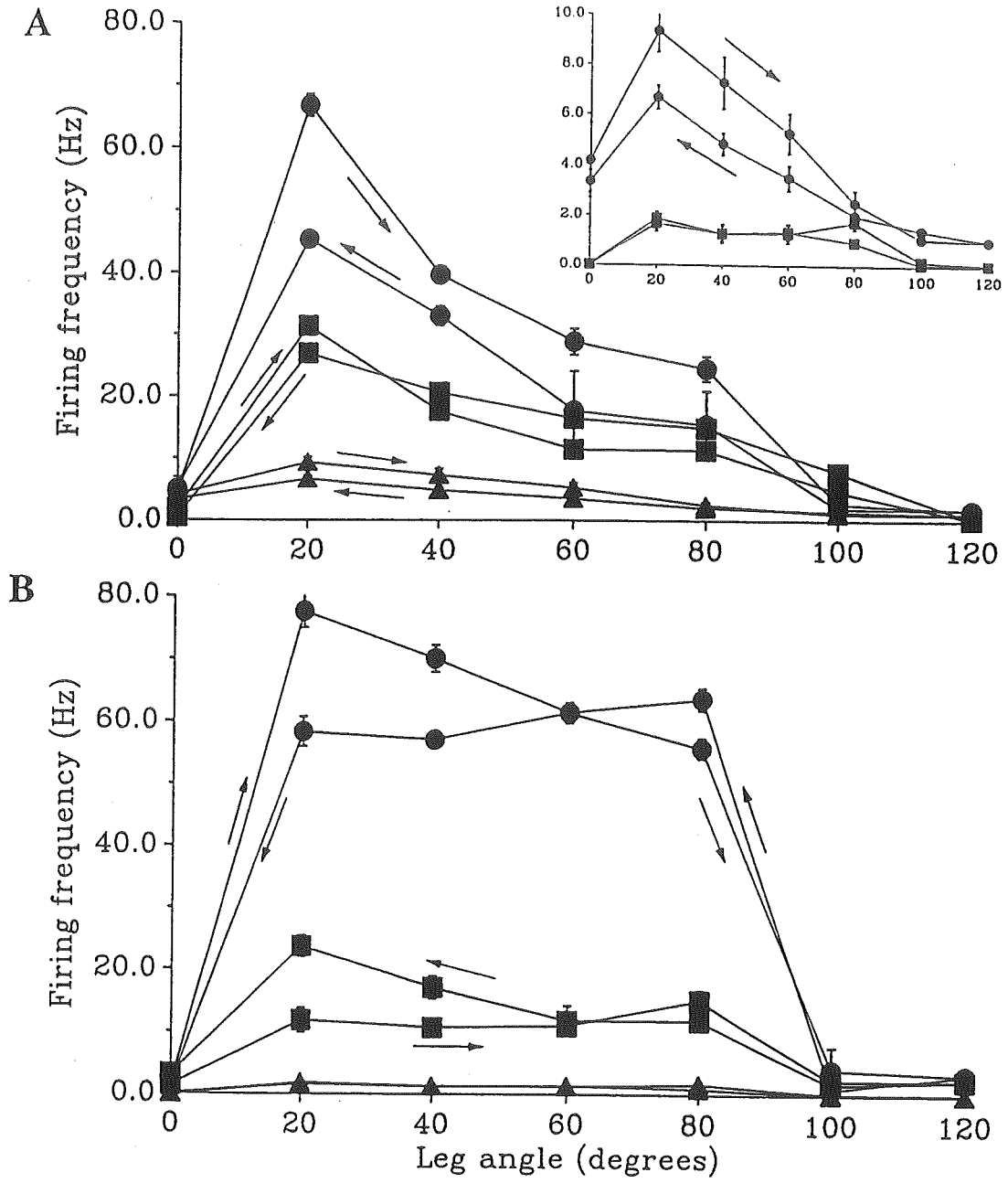


Figure 2 A,B. Tonic neurones with maximal firing at 20°. **(A)** Units with a gradual decline in firing between 20 an 120°. *Circles* = $P^{20}V^{+}?A^{+}$, *squares* = $P^{20}?A^{+-}$, *triangles* = $P^{20}V^{-}?A^{-}$. **(B)** Units with a broad plateau of maximal firing between 20 and 80°. *Circles* = $P^{20}V^{+}?A^{+}$, *squares* = $P^{20}V^{+}?A^{-}$, *triangles* = $P^{20}?AS$. **(Inset)** The lowest curves from A and B plotted on an expanded vertical scale.

These peaks were always present only during holds following ramps in one direction, with holds following ramps in the other direction eliciting very few spikes.

Two neurones with maximum firing rates of less than 10 Hz had low firing frequencies at leg angle less than 60°, reached a peak at 60°, and had maintained

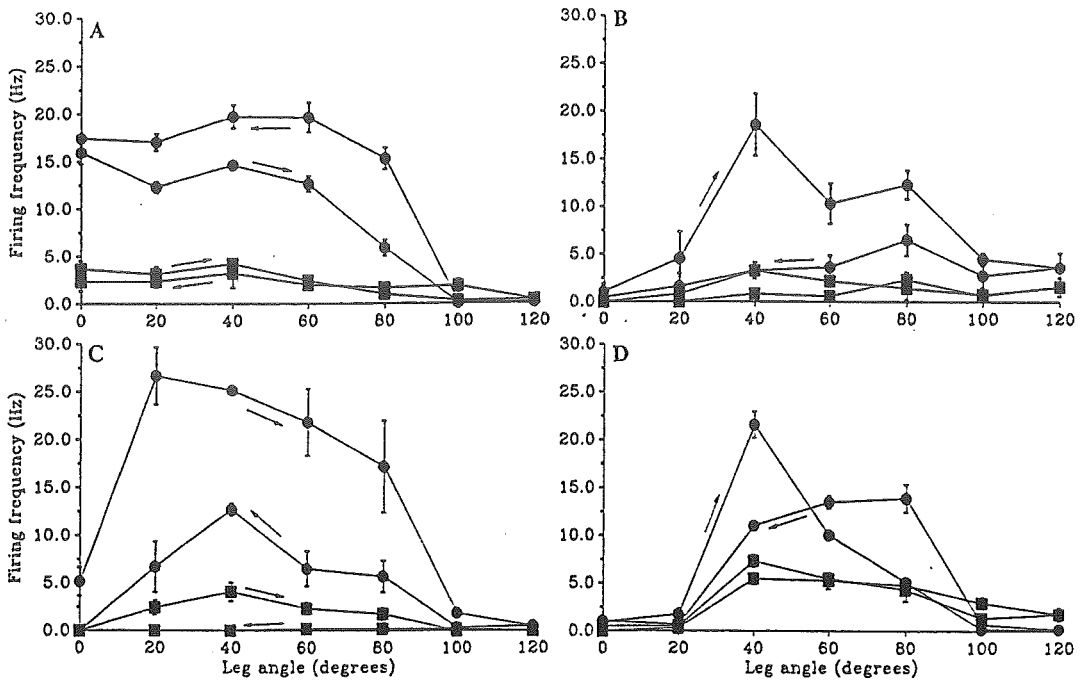


Figure 3 A-D. Neurones with tonic maxima at 40°. (A) Some units had a gradual change in firing (lower curve), while others had a sharp decline between 60 and 100° (upper curve). *Circles* = $P^{40}?A^{+}$, *squares* = $P^{40}V^{-}?A^{-}$. (B) Most units had reduced firing at both extremes of leg movement. *Circles* = $P^{40}V^{-}?A^{-}$, *squares* = $P^{40}V^{-}$. (C) Comparison of hysteresis. Both units $P^{40}V^{-}?A^{-}$. (D) Units with hysteresis causing the response curves to cross over. *Circles* = $P^{40}?A^{+-}$, *squares* = $P^{40}V^{-}?A^{-}$.

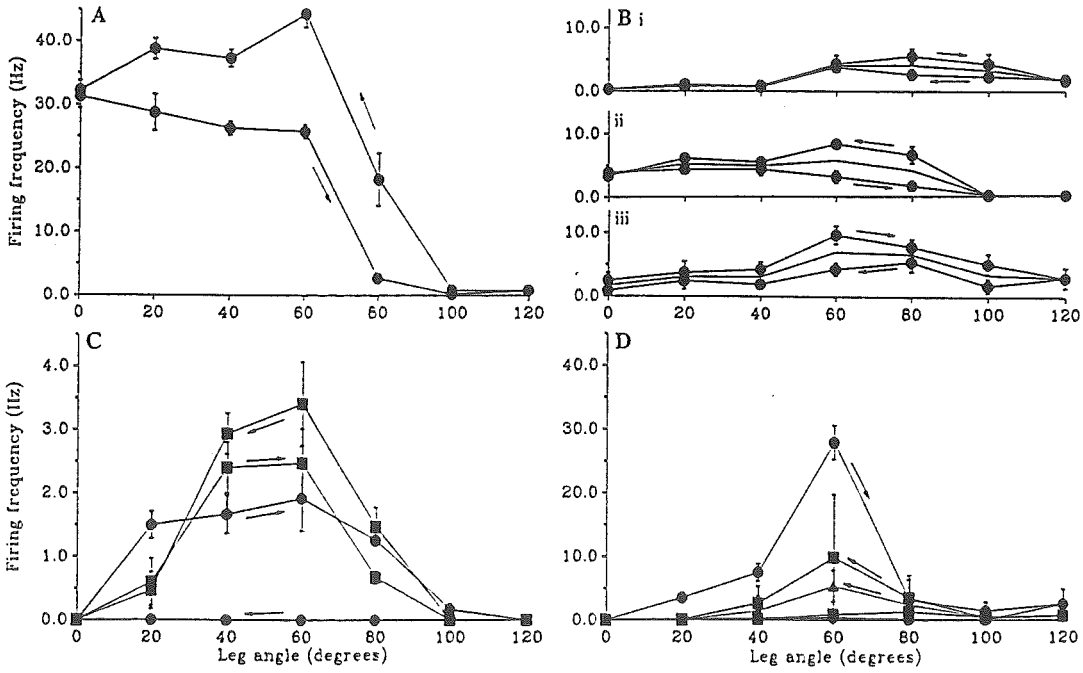


Figure 4 A-D. Tonic neurones with firing maxima at 60°. (A) $P^{60}?A^{?}$ unit with sharp reduction in firing between 60 an 100°. (**Bi,iii**) Units with elevated firing between 60° and 120°. *Bi* = $P^{60}V^{-}$, *Biii* = $P^{60}V^{-}?A^{-}$. (**Bii**) $P^{60}V^{+}?A^{+}$ unit with elevated firing between 0 and 60°. (C) Comparison of hysteresis. *Circles* = $P^{60}V^{-}$, *squares* = $P^{60}V^{-}?A^{-}$. (D) Units with narrow mid-range peaks. *Circles* = $P^{60}V^{-}?A^{-}$, *squares* = $P^{60}?A^{+}$, *triangles* = $P^{60}?A^{+-}$.

high firing between 60° and 120° (Fig. 4Bi,iii). No neurones with tonic maxima at angles less than 60° had maintained high firing at more extended angles. Other P^{60} neurones showed the reverse (higher maintained firing when the leg was flexed than when it was extended: Fig. 4Bii), and were therefore more similar to the response illustrated in Fig. 1A, 3A, and 4A.

Maximum at 80 or 100°. Only 3 neurones had maximum firing at 80° (Fig. 5), while 2 reached their maximum at 100° (Fig. 6). One of the former fired at about 20 Hz for all leg angles between 0 and 80°, but fired at a lower rate (10 Hz) at more extended leg angles (triangular symbols in Fig. 5). It was thus a similar response to the "plateau and sharp drop-off" seen for other tonic neurones. Two neurones had a different response: at 0° they fired at less than 5 Hz, then increased steadily to reach peak firing at 80°. At greater leg angles the firing rate was reduced (circles, squares in Fig. 5). The maximum firing rate of one of these neurones was 60 Hz, while the other only reached 5 Hz.

One of the 2 neurones with tonic maxima at 100° (Fig. 6) had a similar response to those just described: it fired at less than 5 Hz when the leg was set at 0-20°, increasing steadily to reach a peak of approximately 20 Hz at 100°. The

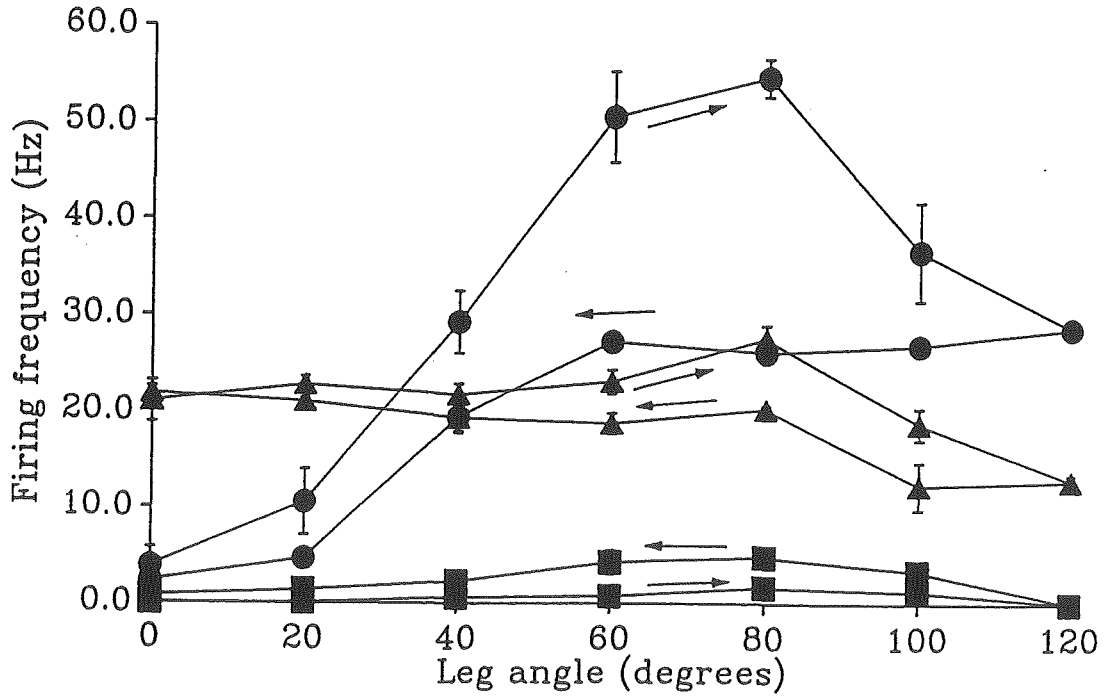


Figure 5. Tonic neurones with maximal firing at 80°. *Circles* = $P^{80}V^{-}$, *squares* = $P^{80}V^{-}?A^{-}$, *triangles* = $P^{80}V^{+}$.

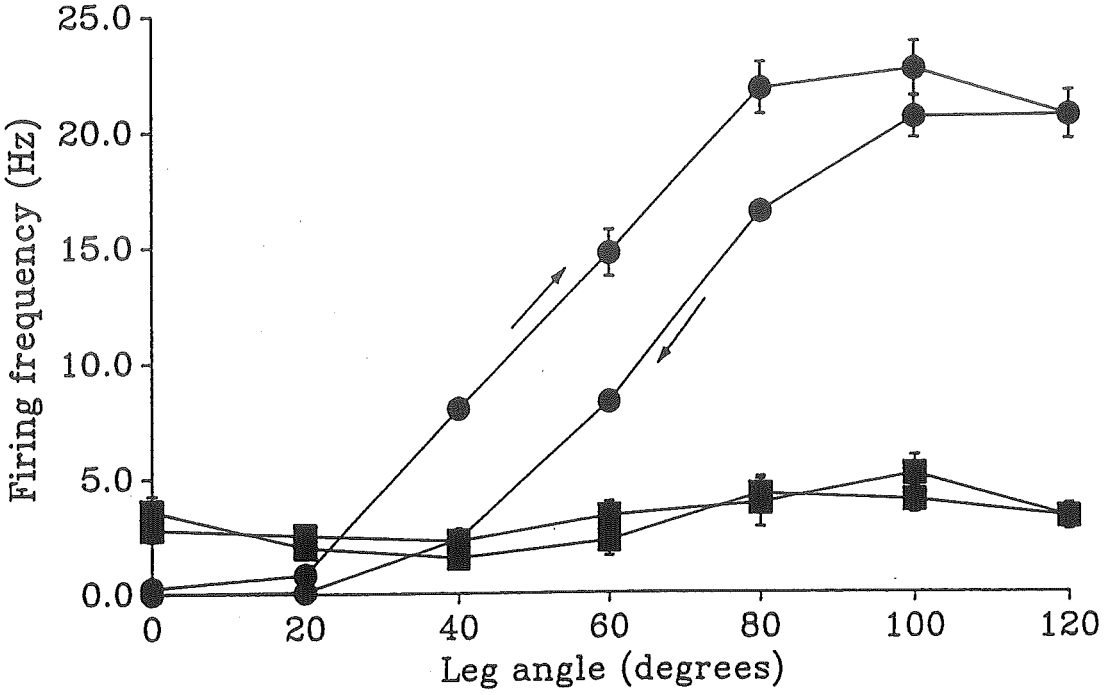


Figure 6. Neurones with tonic maxima at 100°. *Circles* = $P^{100}V^{+}$, *squares* = $P^{100}V^{-}$.

firing rate decreased very slightly at 120° (circles in Fig. 6). The other P^{100} neurone (squares in Fig. 6) only had a weak position sensitivity with a maximum of 5 Hz at 100°. It fired more slowly at 120° (4 Hz), and at more flexed leg angles, reaching a minimum of 2 Hz at 40°. Between 40 and 0° the firing rate increased to 3 Hz, giving the overall curve 2 peaks. This neurone exhibited very little hysteresis.

Maximum at 120°. The maximum firing frequency attained by P^{120} neurones ranged between 3 and 40 Hz (Fig. 7A,B). In addition, the range of leg angles eliciting a tonic response varied markedly between neurones (Fig. 7A-C). In order to highlight this, all the P^{120} responses recorded are presented together in Fig. 7B (values from the extension and flexion sides of the ramp-and-hold stimulus have been averaged for each neurone). In Fig. 7C the same data are plotted on a log scale to emphasise the different response ranges. It is clear that some neurones have response ranges restricted to leg angles of 80-120°, while others respond over the entire range of leg angles. There appears to be a continuum of response width, rather than a separation into distinct response groupings.

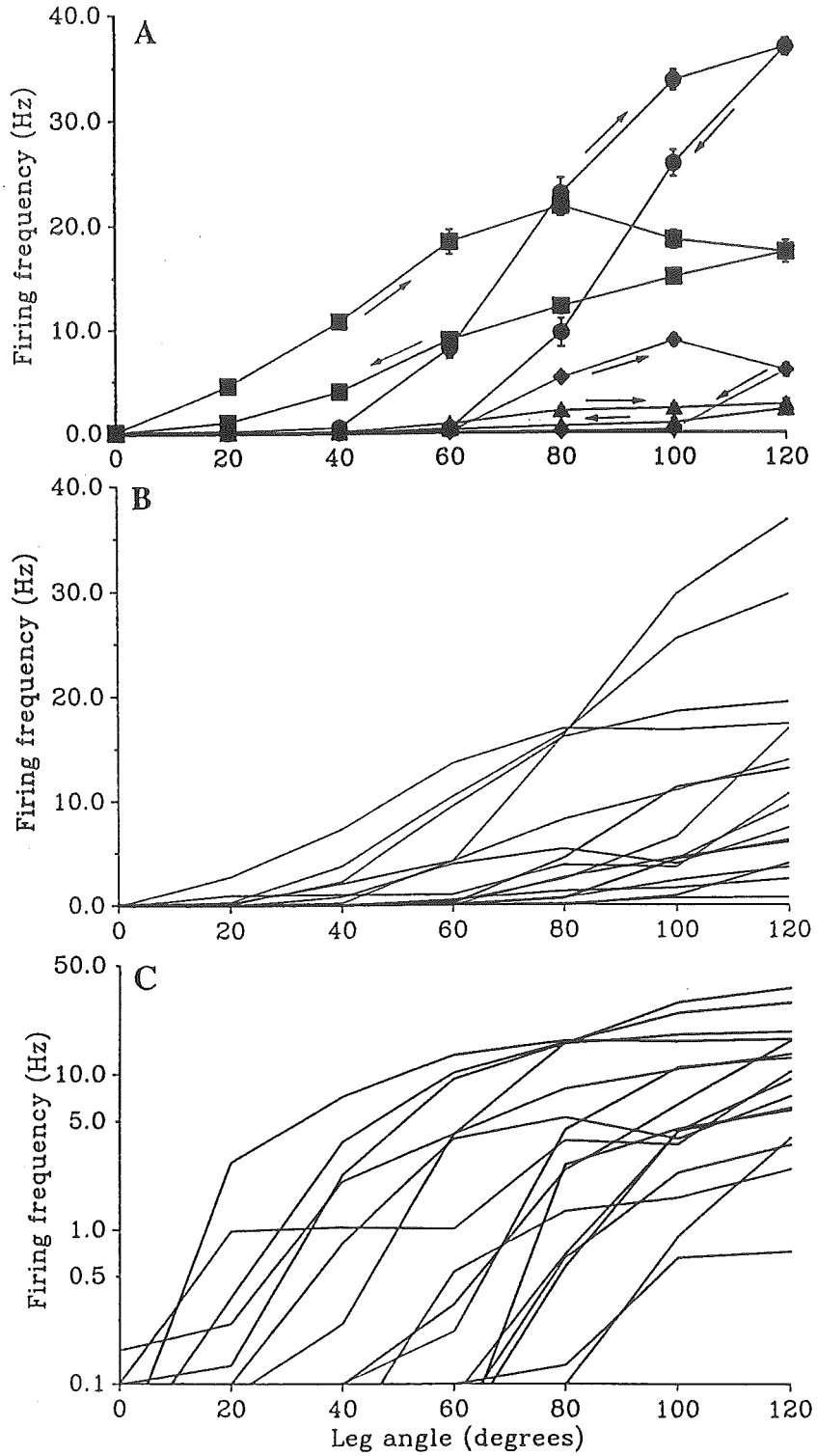


Figure 7 A-C. Neurones with tonic maxima at 120°. (A) Four neurones illustrating response ranges and hysteresis. *Circles, squares, triangles* = P120V-?A-, *diamonds* = P120V-. (B) Selection of units to chosen illustrate response curves. (C) Same data as B on a log scale to emphasise the different response ranges.

Other responses. Several neurones had a tonic maximum at a relatively flexed angle, and a second, smaller, increase in firing frequency at 120° (Fig. 8). This secondary increase is large when compared to the size of the Standard Error bars, and therefore appears to be real. The phasic component of some of these neurones also showed a secondary peak near 120° (lower curve in Fig. 8 is from the same animal as that represented in Fig. 14B).

Several phasic-tonic neurones had erratic firing at most static leg angles (Fig. 9: note the large Standard Errors). This firing was usually caused by movements of tracheae or muscles in the distal femur distorting the FCO apodeme. These neurones were not classified as 'tonic'.

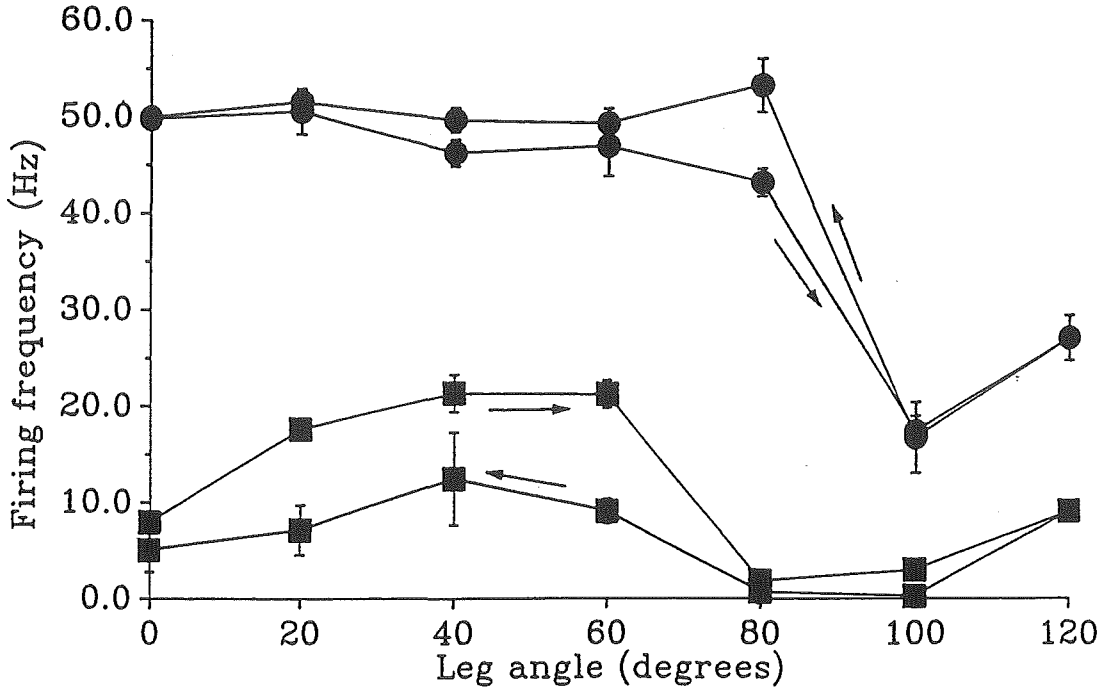


Figure 8. Tonic neurones with elevated firing at both extremes of leg movement. *Circles* = $P^{20}V^{+}$, *squares* = $P^{60}V^{-}?A^{-}$.

Phasic (velocity) responses

Range fractionation may occur in either (or both) of 2 aspects of velocity sensitivity. These are: (1) the range of leg angles over which neurones respond at a given velocity; and (2) the range of velocities to which different neurones respond. Most of the figures in this section are 'families' of curves representing the firing

frequency elicited by different velocity movements in different parts of the leg's arc of movement. They therefore include information about both aspects of the response. Each line represents a single repeat of one complete ramp-and-hold stimulus so error bars cannot be calculated.

It is important to remember that the firing frequencies shown in my graphs are calculated from the number of spikes per ramp. Because all ramps were 20° steps, higher velocity ramps were shorter in duration. This means that spike frequencies calculated for these shorter (faster) ramps will be derived from fewer spikes, and often exhibit greater variation. For example, a neurone could fire 10 spikes at 50 Hz in response to a $100°s^{-1}$ movement, but only 4 spikes 200 Hz during a $1000°s^{-1}$ movement.

I have pooled all the neurones possessing a velocity sensitive component irrespective of any additional responsiveness to position or acceleration. I first examine the response ranges of extension sensitive and then flexion sensitive neurones, before going on to describe the relationship between tibial velocity and firing frequency.

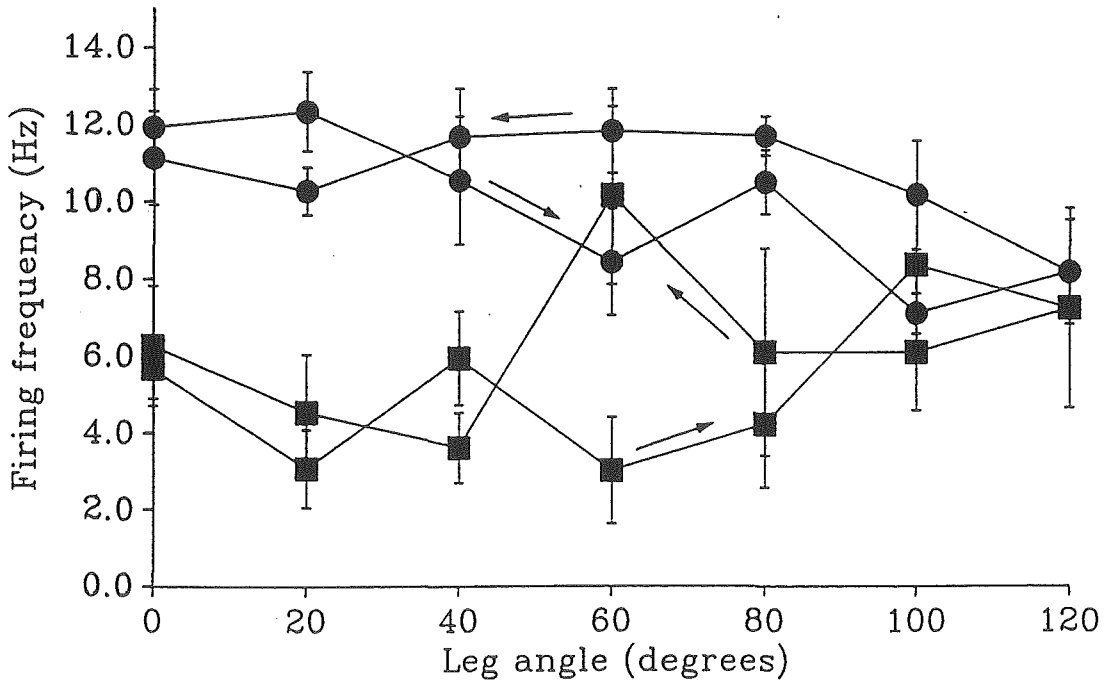


Figure 9. Neurones with ongoing activity unrelated to leg angle. These neurones were not classified as 'tonic' because the firing appeared to be caused by tracheal movements. Both neurones classified V^+.

Extension sensitive neurones. Femoral chordotonal organ neurones which responded in my experiments to the velocity of leg extension (V^-) must be relaxation sensitive (tibial extension = apodeme ligament relaxation). The flexor strand was held at a fixed angle and could not have contributed to this response.

The majority of V^- neurones (45/72) responded most strongly to extensions close to 0° (e.g., the 0-20° step of a ramp-and-hold stimulus: Fig. 10). Higher leg velocities caused increased firing at all angles (Fig. 11). At the highest velocity tested ($1000°s^{-1}$) the frequency of action potentials during the 0-20° ramp varied between neurones within the range 100-350 Hz (note: for a 0.02 s ($1000°s^{-1}$) ramp, a single spike equates to an instantaneous firing frequency of 50 Hz). Some of these neurones were very sensitive: they responded to movements at $10°s^{-1}$ with firing frequencies of 20-30 Hz (slower movements were not tested).

The range of leg angles over which any one neurone responded sometimes depended on the velocity of movement: in all such cases a higher velocity caused the neurone to respond over a wider range of angles. This phenomenon occurred in the responses of virtually all phasic neurones which had restricted ranges at

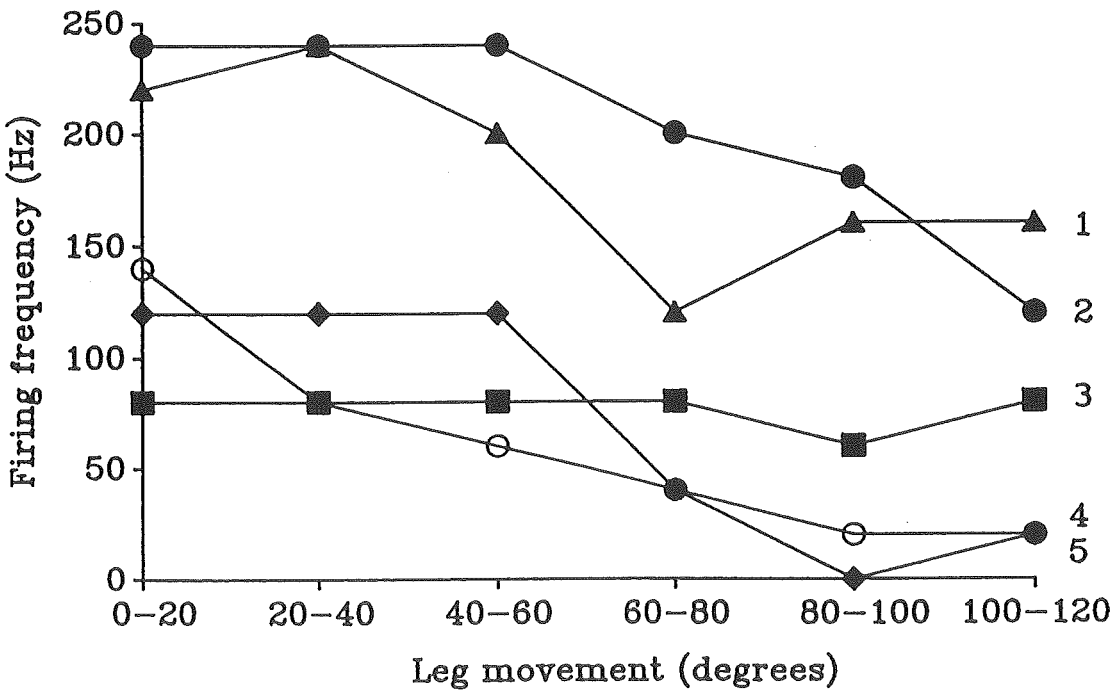


Figure 10. Extension sensitive neurones with maximal responses close to 0°. 1 = V^-, 2 = $P^{40}V^-$, 3 = $P^{120}V^-$, 4 = $P^{40}V^-?A^-$, 5 = V^-.

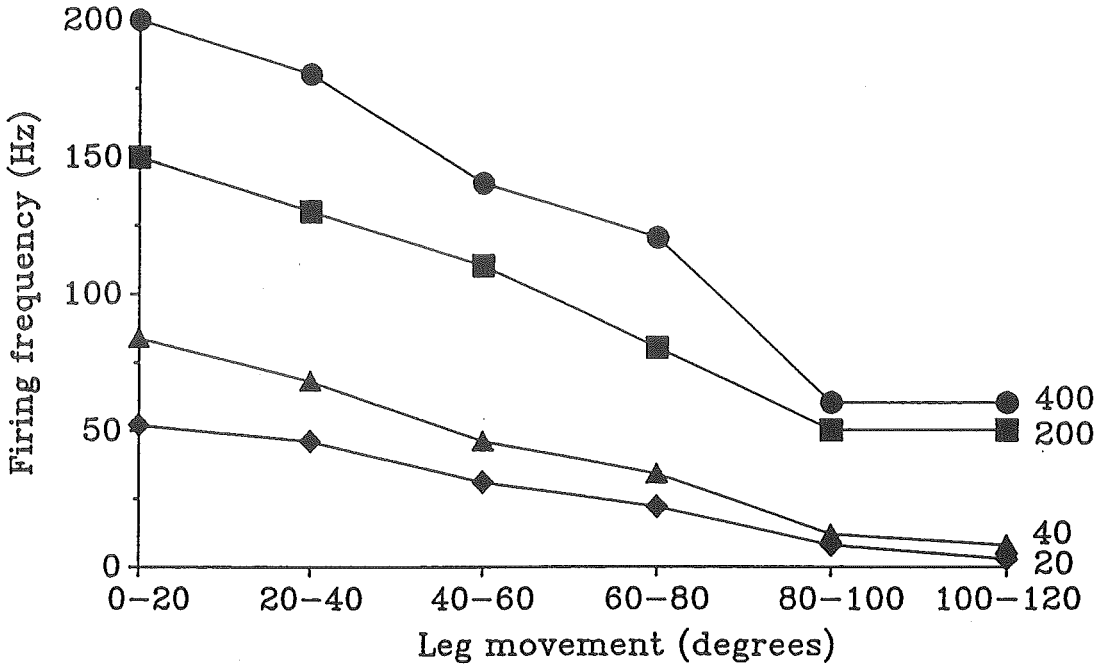


Figure 11. $P^{40}V^{-}?A^{-}$ neurone stimulated by different velocities of tibial movement (numbers to right (°s-1)).

lower leg velocities, and can be seen in many of the figures of this and the next section.

Neurones with V^{-} maxima at 0° included pure velocity units (V^{-}), and neurones with mixed position, velocity, and acceleration responses (e.g., $P^{0}V^{-}$, $P^{80}V^{-}?A^{-}$). Phasic-tonic neurones had tonic maxima in the range 0 to 120°.

Twenty two V^{-} neurones were most sensitive to movements at mid-angles, and had reduced firing at both extremes of leg movement (Fig. 12A,B). The maximal firing frequencies of these neurones ranged from 50 to 300 Hz (for a 1000°s^{-1} movement), with most firing at or below 150 Hz. The neurone represented in Fig. 12B fired at 300 Hz during a 400°s^{-1} ramp, but was not tested at any higher velocities. At low velocities of movement (e.g., 20°s-1: lowest curves in Fig. 12A) these neurones often did not fire at all at extreme leg angles. At higher velocities their firing frequencies increased, and the range of leg angles in which extensions elicited spikes widened (other curves in Fig. 12A). At 1000°s^{-1} some neurones still did not fire at 0° (Fig. 12A). Other neurones fired in response to movements at all leg angles (Fig. 12B). Neurones with mid-angle velocity maxima included both pure velocity units (V^{-}) and units with mixed position, velocity, and acceleration responses (e.g., $P^{120}V^{-}$, $P^{60}V^{-}?A^{-}$). All the neurones in this group with a tonic component had their tonic maximum between 60 and 120°.

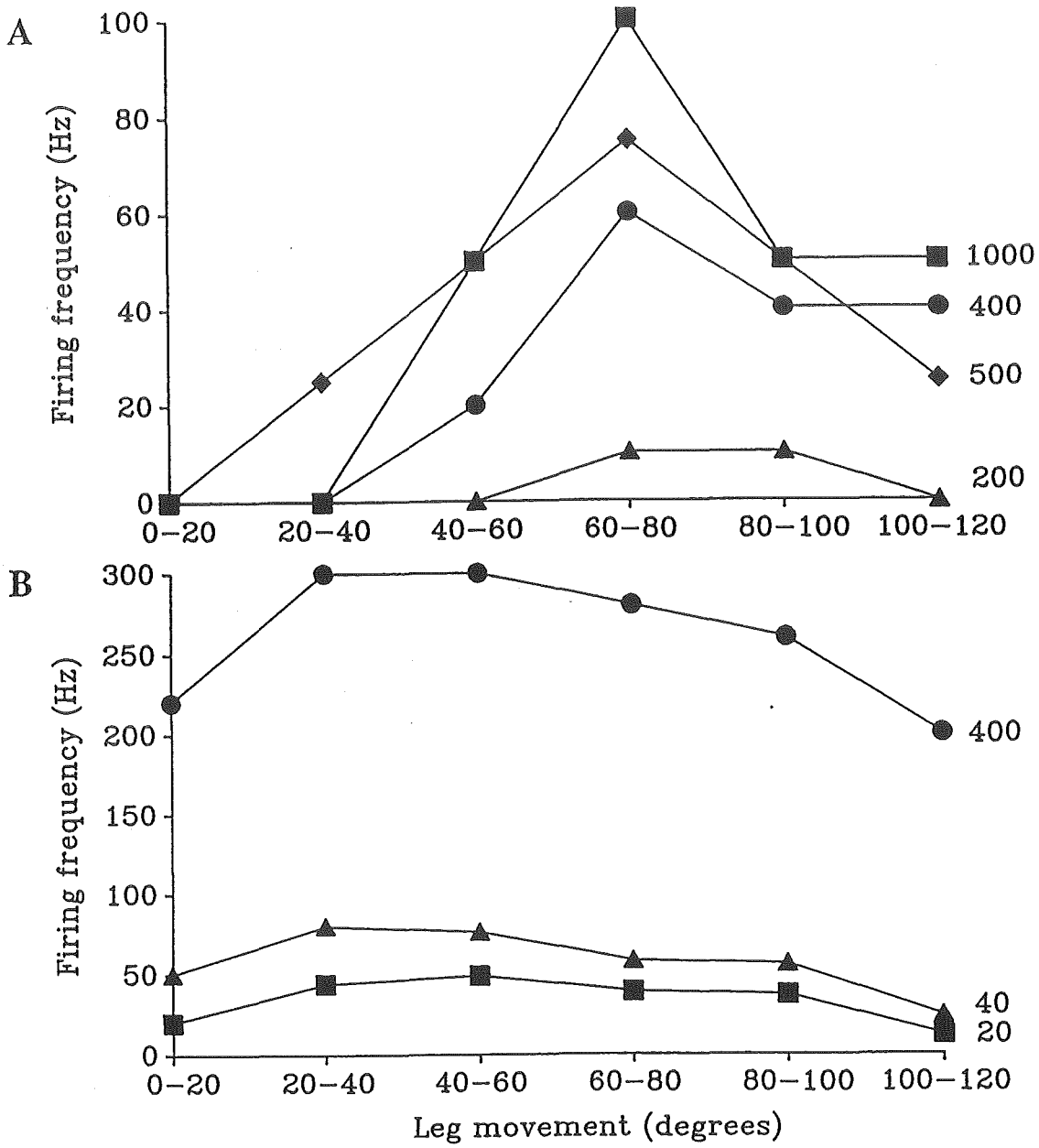


Figure 12 A,B. Extension sensitive neurones with maximal responses to movements at mid-angles. **(A)** V^- unit stimulated at the velocities indicated to the right ($°s^{-1}$). **(B)** $P^{60}V^-$ unit stimulated at velocities of 20 to 400$°s^{-1}$.

Three extension-velocity sensitive neurones had maximal firing frequencies at 120° (Fig. 13A,B). For 1000$°s^{-1}$ movements, their maximum firing rates were 50 Hz (V^-), 100 Hz ($P^{120}V^-$: Fig. 13B) and 150 Hz ($P^{120}V^-$: Fig. 13A) respectively.

Seven neurones had unusual responses: they fired most in response to extensions close to 0°, but also had a secondary peak of sensitivity at the 100-120° step of a ramp-and-hold stimulus (Fig. 14A,B). These neurones had maximal

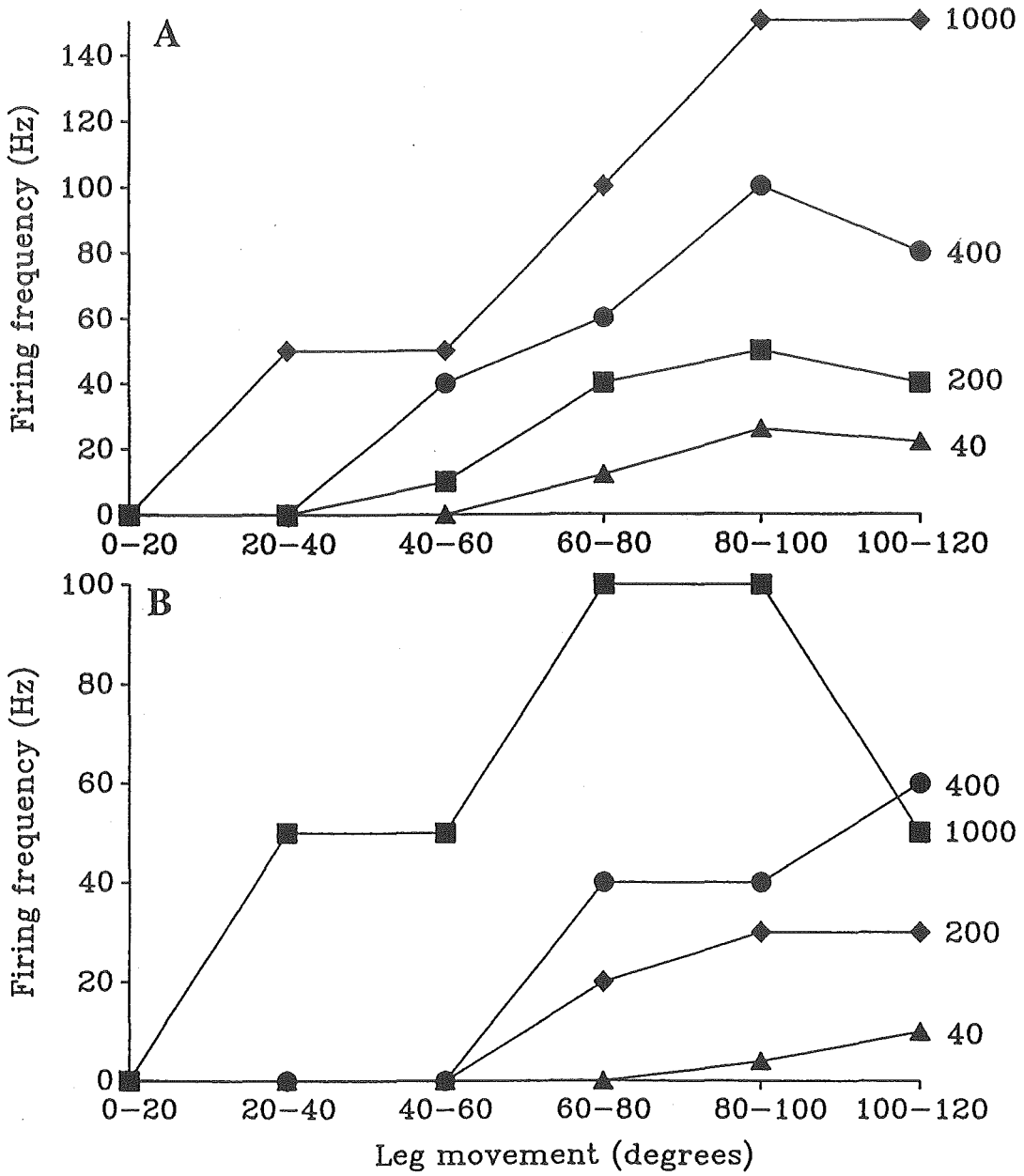


Figure 13 A,B. Effect of different stimulus velocities on firing of extension sensitive neurones with maximal phasic responses near 120°. Velocity of movement is indicated in degrees per second on the right side of the figure. Both examples are $P^{120}V^{-}$ units.

firing frequencies (for 1000°s^{-1} leg extensions) ranging between 150 and 300 Hz. All of these neurones were of the response type $P^{x}V^{-}?A^{-}$, where *x* is in the range 40-120°. Other neurones with a $P^{x}V^{-}?A^{-}$ response did not show this secondary peak.

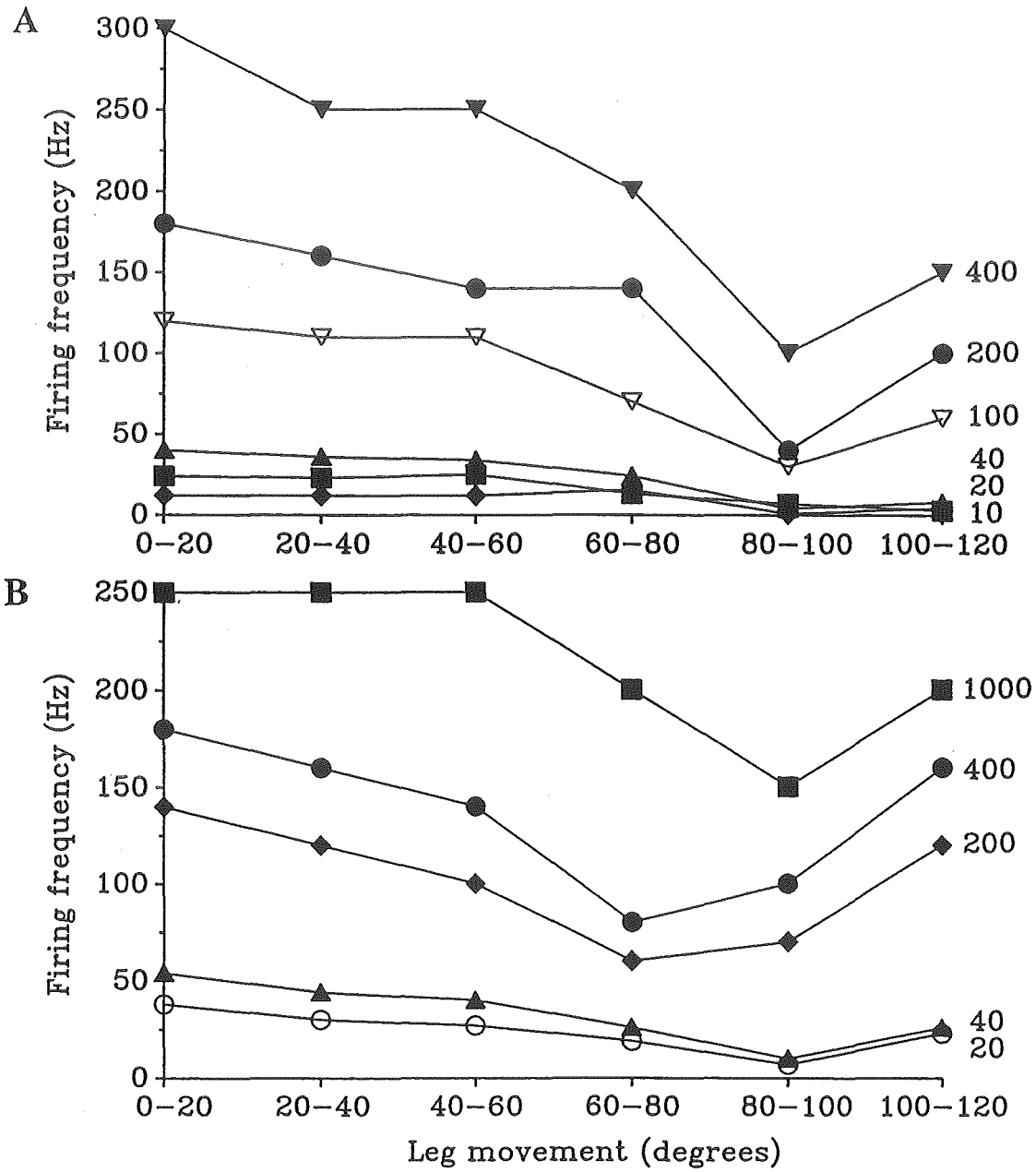


Figure 14 A,B. Extension sensitve neurones exhibiting increased phasic firing at both extremes of leg movement for all velocities tested (numbers to right ($°s^{-1}$)). Both neurones classified $P^{60}V^{-}?A^{-}$.

Flexion sensitive neurones. Neurones which responded to stretch of the apodeme ligament (tibial flexion) responded to higher velocities with higher firing frequencies (n = 65). At higher velocities these neurones often fired in response to movements in a greater range of leg angles.

Forty-four flexion-velocity sensitive neurones responded most strongly during flexions close to 0° (Fig. 15). Some neurones of this type fired more spikes at each step successively closer to 0°, and hence had response curves which did not

flatten out (Fig. 16A). Other neurones did reach a plateau over the last 2-3 steps (Fig. 16B). Of the former type, 21 were purely phasic, and 3 were phasic-tonic (P^0V^+). Eight of the latter (plateau) type were purely phasic, while 10 had a P^0V^+ response. This difference is statistically significant (Chi^2: P = 0.0028, 1 df). The only $P^{100}V^+$ neurone recorded fell into this latter group. Maximal firing frequencies (20-0° step, 1000°s^{-1}) ranged from 100 to 350 Hz, with most in the range 100-200 Hz. Flexion-velocity sensitive neurones with maxima at 0° included some with the narrowest phasic response ranges observed (e.g., right-most curves in Fig. 15, and Fig. 17). In extreme cases, firing could only be elicited in the last step (20-0°) at high velocities (Fig. 17 and inset). Generally, neurones which reached a plateau near 0° had wider response ranges at all velocities than did neurones which did not reach a plateau.

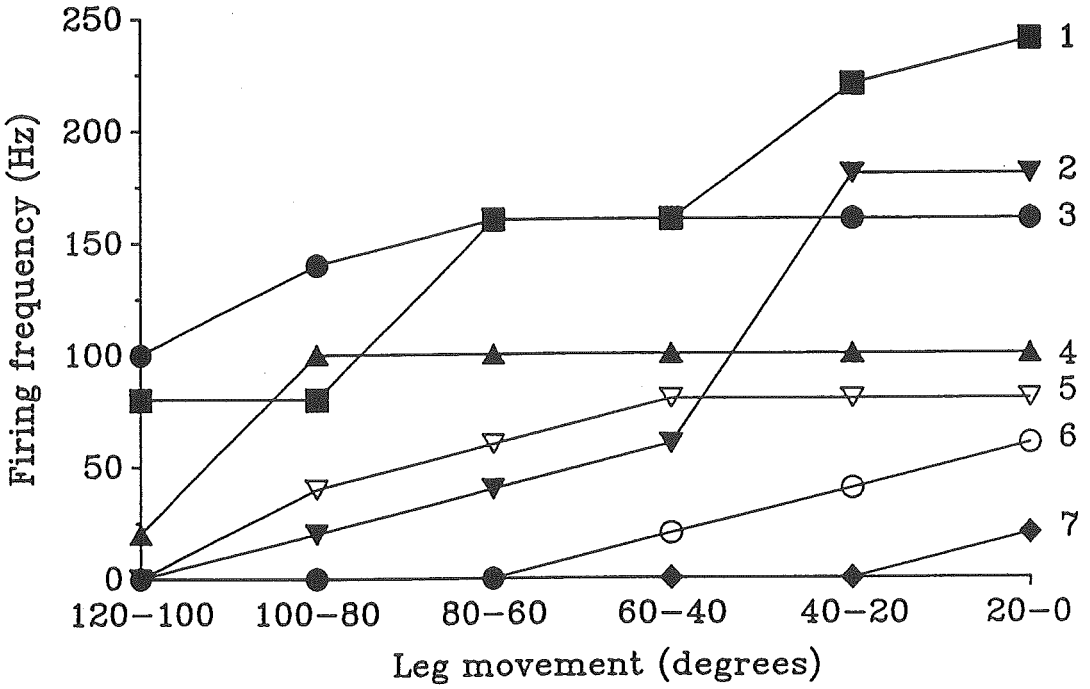


Figure 15. Flexion-velocity sensitive neurones with maximal phasic responses near 120°. Each curve represents a different neurone. All neurones were stimulated at 400°s^{-1}. 1,3-7 = V^+, 2 = P^0V^+.

Many flexion sensitive neurones had very wide response ranges, with constant firing rates across the full range of leg angles at a given velocity (Fig. 18A,B). Some of these neurones had the most sensitive phasic (velocity) responses recorded (e.g., neurone in Fig. 18A fired at 50 Hz during movements at 6.7°s^{-1}). Slower movements were not tested methodically, but this neurone at least, responded to movements as slow as 0.5°s^{-1}. Both purely phasic and phasic-tonic neurones were included in this group. Tonic maxima were in the range 0-80°.

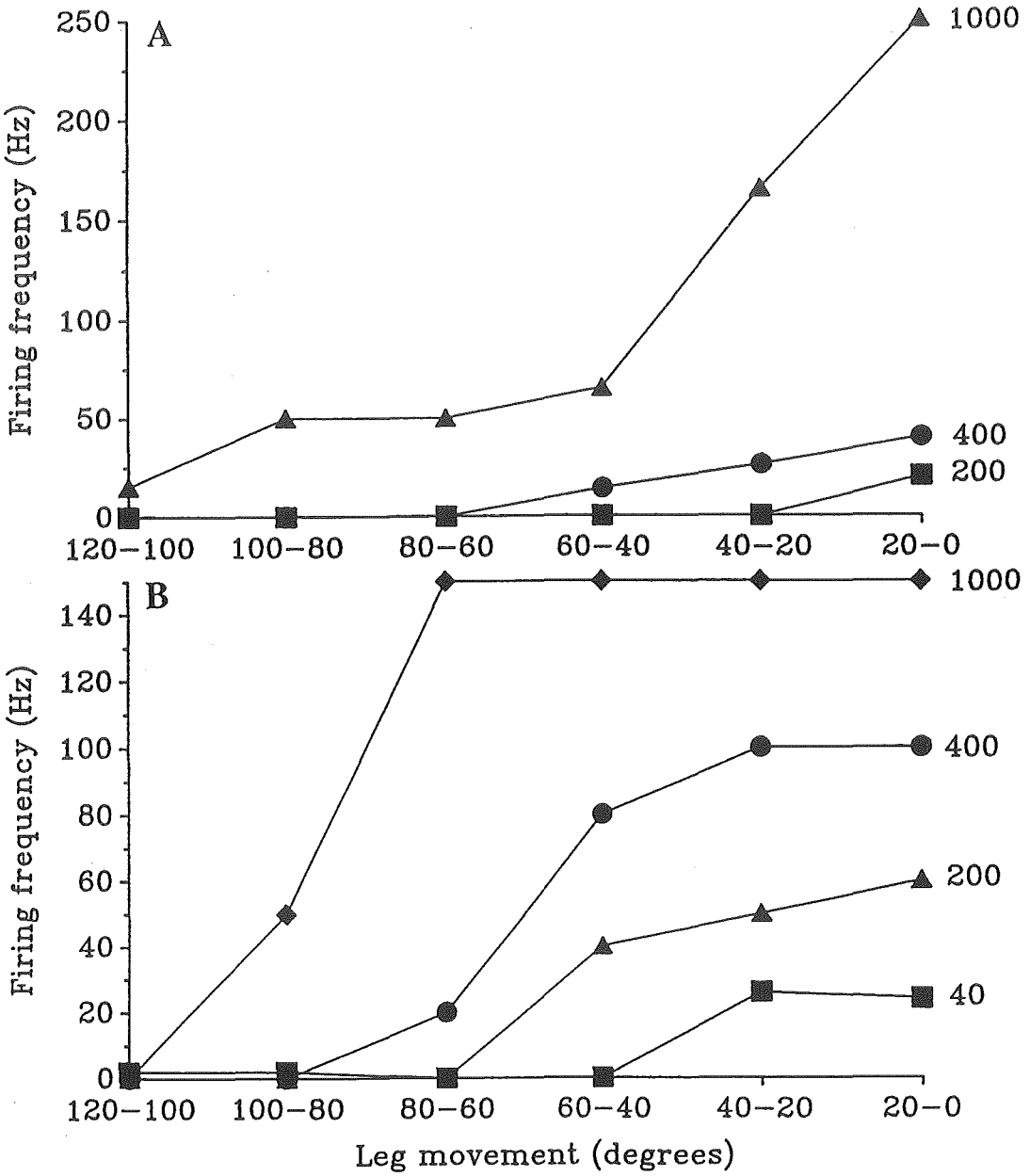


Figure 16 A,B. Flexion sensitive neurones with maximal phasic firing near 120°. (A) V^+ unit which did not reach a plateau. (B) P^0V^+ unit which responded with similar firing frequencies to movements over a wide range of leg angles. The effect was exaggerated at higher stimulus velocities.

Only 5 neurones had maximum sensitivities to flexions at mid-angles. These responses were rather weak (Fig. 19). The maximum firing frequencies (1000°s^{-1} ramp) ranged between 150-250 Hz. Two neurones were not tested at 1000°s^{-1}: their maximal firing during 400°s^{-1} ramps was 200 or 250 Hz respectively. All these mid-position neurones were phasic-tonic: the tonic maxima ranged from 0-40°.

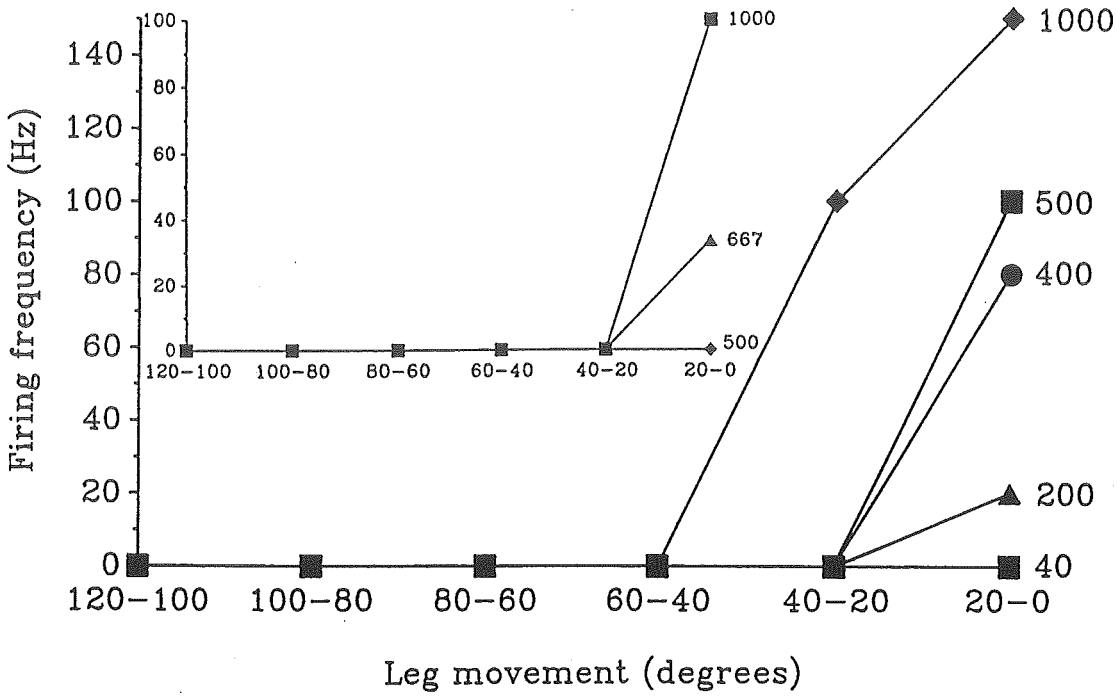


Figure 17. Flexion sensitive neurone with a response range restricted to near 120° for all velocities between 200 and 1000°s^{-1}. **Inset** Similar neurone which only responded during the 20-0° ramp, even when stimulated at 1000°s^{-1}.

Three neurones fired most strongly during ramps near 120°. In 2 cases the responses were rather weak, while the other neurone had a more marked peak, at least at the higher velocities (Fig. 20). In all cases, the maximal firing frequency during a 1000°s^{-1} ramp was 200 Hz. All these neurones had $P^xV^+?A^+$ responses (where x = 0-20). Other neurones with this classification did not have maximal velocity responses at 120°.

Effect of tibial velocity on firing frequency during ramps

All recorded neurones had higher firing frequencies at 1000°s^{-1} than at the next slowest movement tested (usually 400°s^{-1}, but occasionally 500 or 600°s^{-1}) (i.e., firing frequency was never seen to completely saturate). Some neurones did however have different lower thresholds to velocity. The most sensitive neurone responded to movements at least as slow as 0.5°s^{-1}. Another neurone responded to movements at 1000°s^{-1}, but not those at 400°s^{-1}. The majority of neurones responded to all the velocities tested (20-1000°s^{-1}). Neurones which only responded to higher velocities were usually those with narrow response ranges near either 0° or 120°. I did not attempt to determine velocity thresholds for the majority of neurones, but it is interesting to examine the above-threshold

response to velocity. Twenty-three neurones chosen from 6 categories of velocity sensitive units (including wide and narrow range V^+ and V^- types, and V^- mid position types) were examined in detail (4 units from each type except wide range V^+ (3)). Firing rates were plotted against ramp velocity for the ramp which elicited the greatest firing (i.e., 20-0° ramp for V^+ units which fired most as the leg approached 0°). For the wide range units, ramps in 3 ranges of leg angle were analysed: 0-20, 60-80, and 100-120.

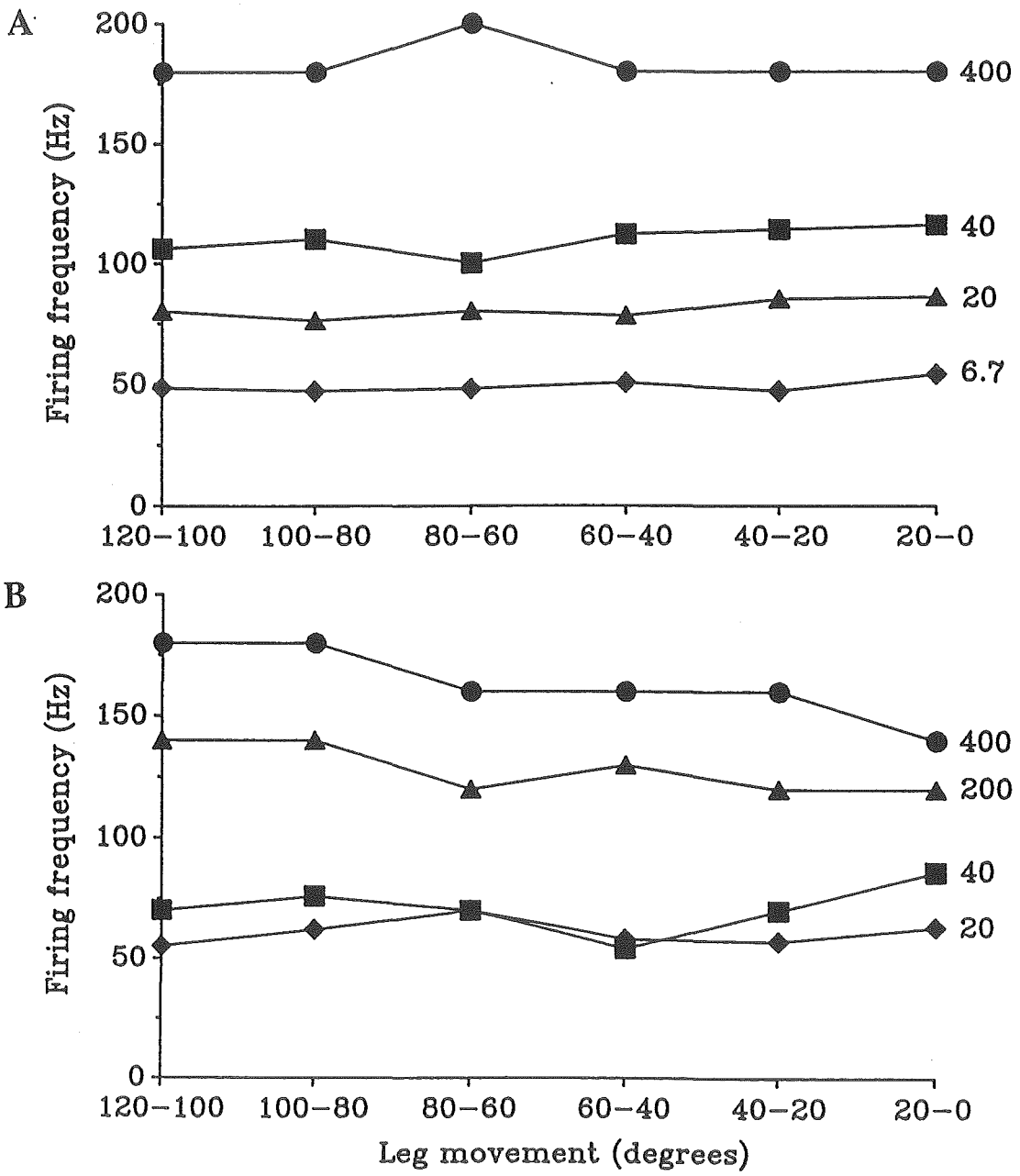


Figure 18 A,B. Flexion sensitive neurones which responded with uniform firing across the full range of leg angles. A = V^+. B = P^0V^+.

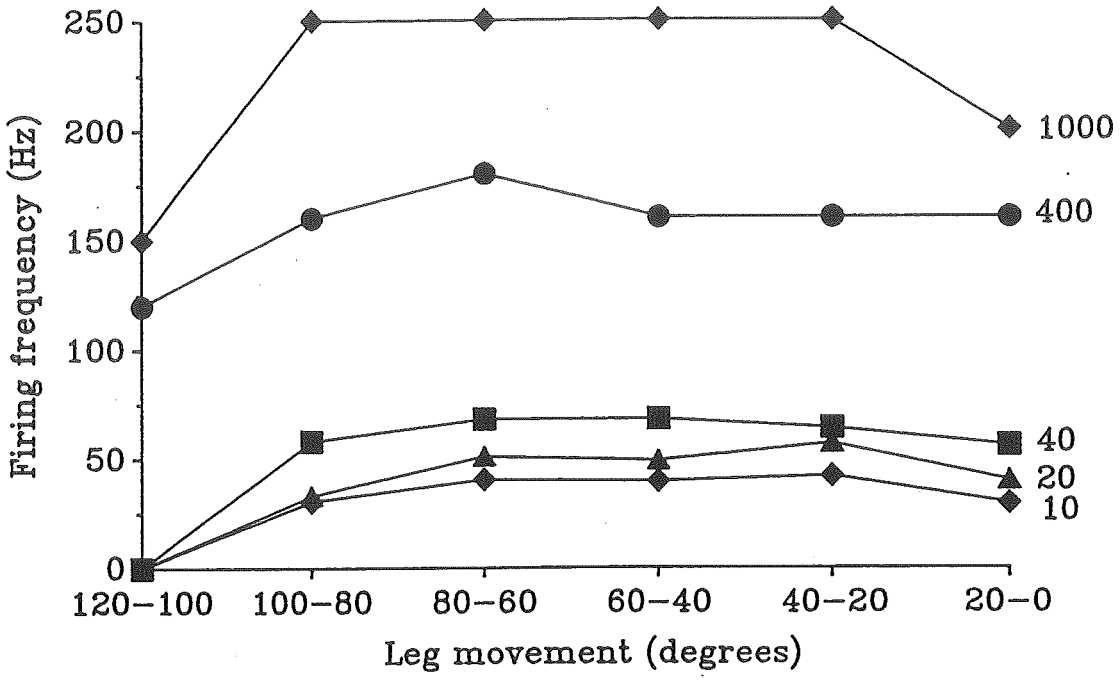


Figure 19. Flexion sensitive neurone ($P^{20}V^{+}$) with maximal phasic firing at mid-angles. The velocities used are indicated at the right ($°s^{-1}$).

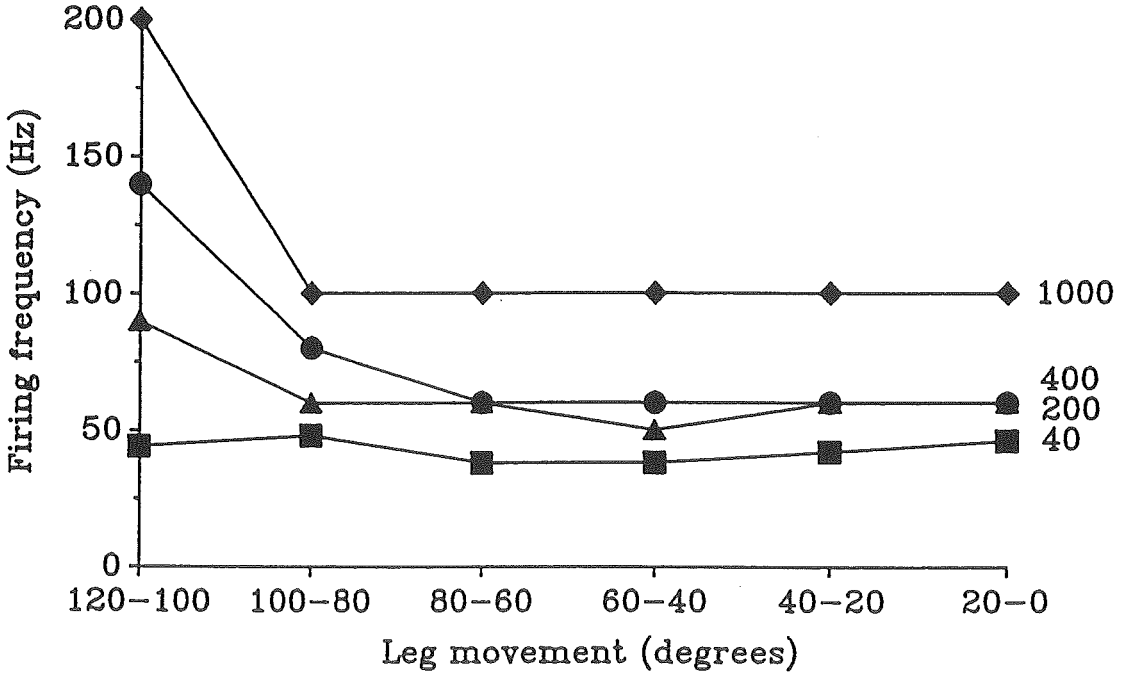


Figure 20. Flexion sensitive neurone ($P^{0}V^{+}?A^{+}$) which responded most strongly to movements near 120°. The effect was exaggerated at higher velocities.

When plotted on linear axes the majority of neurones exhibited some degree of saturation at higher velocities (e.g., Fig. 21). Although there was some variation both between and within the chosen classes with respect to absolute firing frequency and the shape of the response curve, this did not appear to be systematically related to any other features of response type.

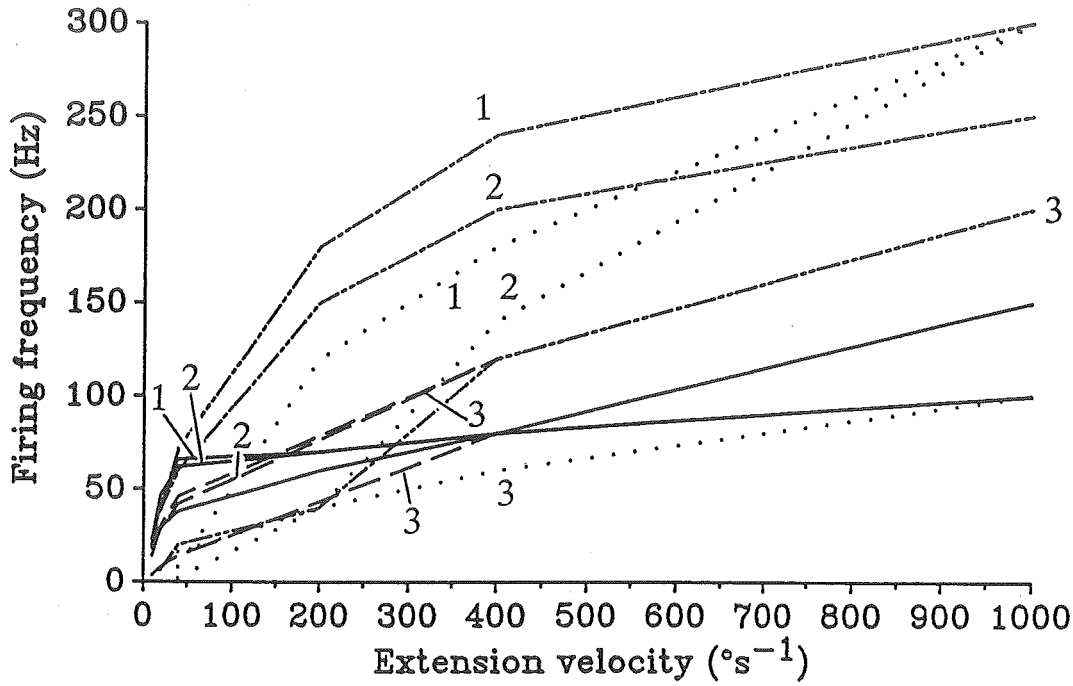


Figure 21. Effect of extension velocity on the firing frequency of 4 wide range, velocity sensitive neurones. Each neurone was tested at 0-20° (a), 60-80° (b), and 100-120° (c). (—·—) = $P^{40}V^-$, (– – –) = $P^{80}V^-?A^-$, (·····) = $V^-?A^-$, (——) = $P^{120}V^-$.

Regressions were calculated for individual neurones from 3 classes: Fig. 22A: V^+ wide (20-0° ramp), Fig. 22B: $V^{-\,wide}$ (0-20° ramp), and Fig. 22C: $V^{-\,mid}$ (60-80° ramp). In this figure, solid and open (dashed) symbols and lines are simply used to help distinguish between neurones: they have no other significance. On average, r^2 values were higher for data plotted on log-log axes than on either linear or log-linear (x-y) axes, indicating that the general form of the regression should be:

$$\log(\text{firing frequency}) = a.\log(\text{ramp velocity}) + b$$

where a is the slope of the regression line, and b is the Y intercept. This equation can alternatively be expressed as:

$$(\text{firing frequency}) = 10^b.(\text{ramp velocity})^a$$

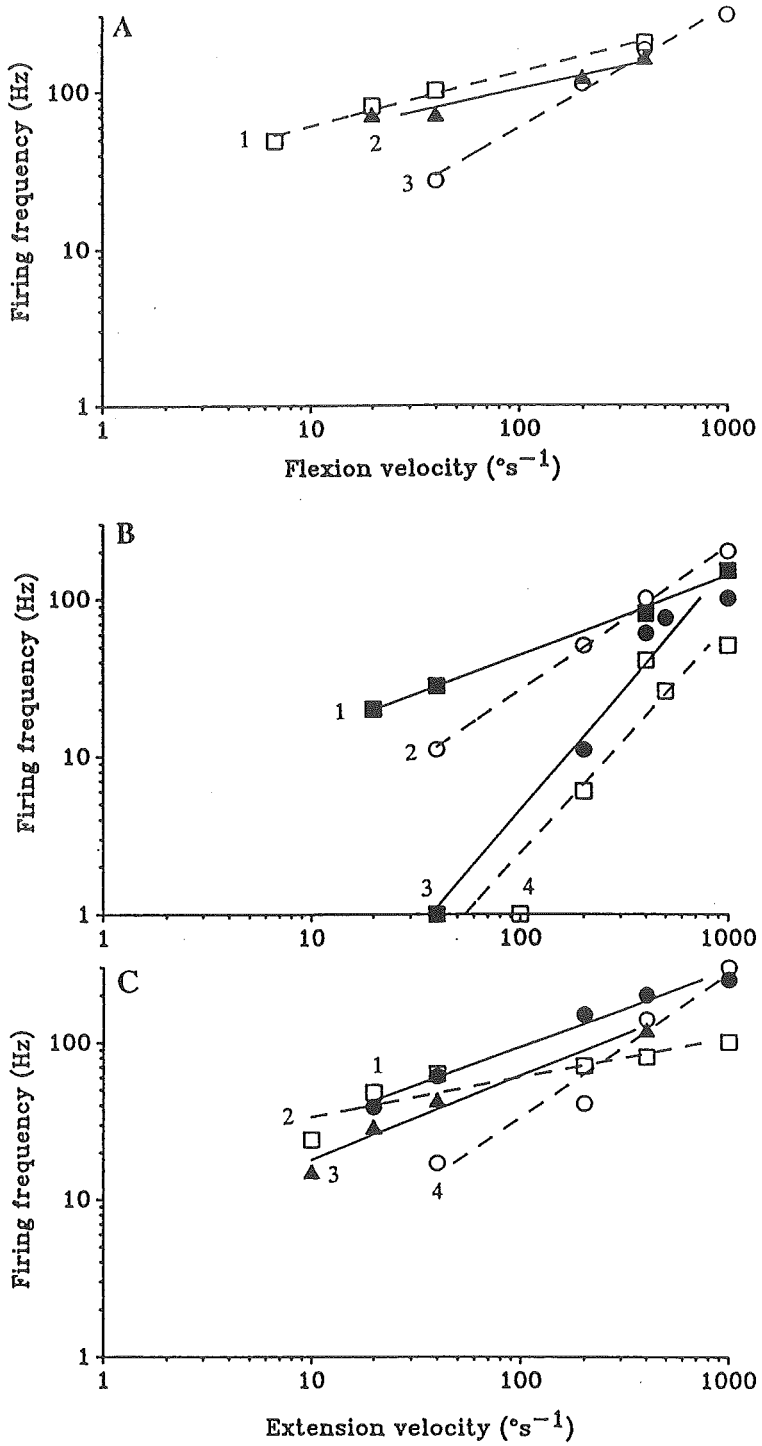


Figure 22 A-C. Effect of tibial velocity on firing frequency of phasic neurones. (A) Wide range flexion sensitive units (20-0° ramp). *Circles* = P^0V^+: logY = 0.76 logX + 0.25, r^2 = 0.988. *Squares* = V^+: logY = 0.337 logX + 1.44, r^2 = 0.987. *Triangles* = P^0V^+: logY = 0.29 logX + 1.44, r^2 = 0.952. (B) Wide range extension sensitive units (0-20° ramp). *Open circles* = $P^{120}V^-$: logY = 0.91 logX - 0.406, r^2 = 0.997. *Closed circles* = V^-: logY = 1.55 logX - 2.44, r^2 = 0.962. *Open squares* = V^-: logY = 1.45 logX - 2.52, r^2 = 0.892. *Closed squares* = $P^{120}V^-$: logY = 0.50 logX + 0.639, r^2 = 0.995. (C) Mid-position extension sensitive neurones (60-80° ramp). *Open circles* = $V^-?A^-$: logY = 0.92 log X - 0.31, r^2 = 0.949. *Closed circles* = $P^{40}V^-$: logY = 0.49 logX + 0.99, r^2 = 0.979. *Triangles* = $P^{80}V^-?A^-$: logY = 0.54 logX + 0.71, r^2 = 0.971. *Squares* = $P^{120}V^-$: logY = 0.25 logX + 1.27, r^2 = 0.820.

The individual regression equations, regression coefficients, and details of each neurone's response type are given in the figure captions

Two neurones in Fig. 22B appear to have steeper response curves than other neurones. It is not possible to statistically test the significance of this difference because each line is derived from only 1 complete ramp-and-hold stimulus (i.e., the errors cannot be calculated). Both these neurones were V^- units, while the other 2 in Fig. 22B were $P^{120}V^-$ units.

DISCUSSION

Range fractionation

Range fractionation clearly does occur in the locust mtFCO, and there are units which could act as labelled lines to signal leg position. Narrow-range units are present almost exclusively at the extremes of leg movement, but a few mid-range units also have restricted ranges (see Fig. 9D). The response ranges of most neurones (both phasic and tonic types) tend to be wide. This contrasts to the crab myochordotonal organ, for example, where only 1 out of 48 flexion sensitive neurones responded over the full range of leg angles (Cohen 1963). The considerable overlap of wide-range neurone's response ranges should allow the CNS to extract accurate positional information about all leg angles, even in the absence of 'mid-position labeled line' units. Neurones with sensitivities restricted to near 0° could act as labelled lines to indicate when the tibia is fully flexed. This information should be important when the animal is preparing for a jump. It would be of considerable interest to investigate the connections of such sensory afferents onto interneurones (such as the M cell) which are known to initiate jumping. The only units with narrow response ranges near 120° were a limited number of position sensitive cells. However, some other position sensitive units have uniformly high firing frequencies between 0 and 100°, and a sudden decline in firing at more extended angles. These neurones also can therefore precisely signal extreme extension.

It is interesting to note that some phasic-tonic neurones had secondary peaks of firing at 120° (Fig. 8,14). In some cases both the phasic and tonic components showed this trend, but in others only one component did so. These neurones appear to be the first examples of U-shaped firing curves in an FCO. Zill (1985a) stated that "phasic units were active in ranges of either extension or flexion but not both".

The lack of neurones with narrow-range extension sensitivity may reflect methodological constraints rather than a physiological difference: it is possible that such units do exist, but are activated by stretch of the flexor strand. In my experiments this ligament was held at a fixed angle of 60°. In addition, the metathoracic leg of *Locusta* can extend as far as 150°, so it is possible that there are units which begin to fire beyond 120° to signal full extension. Zill (1985a) illustrated 1 such unit which only responded between 150 and 170° in *Schistocerca*.

The relationship between tibial angle and FCO apodeme movement is approximately linear between 0 and 120°, but is distorted at more extended angles (See curve for *Schistocerca* in Field and Burrows (1982)). In particular, extensions between 0 and 120° push the apodeme towards the FCO, while further extensions (beyond 120°) tend to pull the apodeme. Thus, both a flexion from 120 to 100° and an extension from 120 to 140° exert a pull of similar magnitude on the apodeme. Some complex mechanism must exist if FCO neurones are to make a distinction between these movements. Field (in press) has shown that the main ligament to the apodeme is multi-stranded, and that different strands are under differential tension. It is possible that this mechanism is responsible for the observation that groups of neurones (connected to different bundles of strands in the ligament) respond differently to the same movement of the apodeme. Clearly, further research is required in this area.

The firing frequencies of many tonic neurones exceed 40 Hz, yet the whole-nerve recordings of locust mtFCO tonic activity at different leg angles presented in Zill (1985a) and Usherwood et al. (1968) have surprisingly low values (maximum < 160 Hz). Theophilidis (1986b) has slightly more higher values for *Decticus* (minimum approx. 230 Hz, maximum 550 Hz). These low rates suggest that only the largest tonic spikes are being detected in extracellular records, and that the recordings made by the latter author were of better quality. This introduces the possibility of considerable bias in the shape of the extracellularly recorded response curve because neurones with different sized axons are likely to have different responses. It is interesting therefore to note that the shapes of the response curves presented by Theophilidis are different to those presented by Zill (1985a) and Usherwood et al. (1968). Considerable caution should be used when interpreting such extracellular recordings. In particular, these extracellular recordings certainly hide the responses of neurones which have mid-position tonic maxima. Although the extracellular recordings fail to represent small spikes, these neurones must still make significant contributions to the information flowing into the CNS.

Hysteresis in tonic responses

Hysteresis in the responses of mtFCO neurones has been discussed by Matheson (1990) and Zill & Jepson-Innes (1988). Mill & Lowe (1972) presented a theoretical model to explain how the CNS could extract accurate positional information from an otherwise ambiguous position response, but this does not seem to have been investigated at the neuronal level. Examples in the present paper show that hysteresis varies greatly between neurones: even between those with otherwise similar responses. Zill (1985a) and Zill & Jepson-Innes (1988) discussed hysteresis in terms of movements away from, and back to the central leg angle because all the tonic units in those studies had maximal firing at the extremes of leg movement. It is therefore interesting to examine the hysteresis of neurones with mid-position maxima reported in the present paper.

Most tonic neurones had a velocity sensitive component (Table 1). At any given leg angle the tonic firing of most flexion-velocity sensitive neurones was higher if the set angle had been approached by a flexion than if the set angle had been approached by an extension (e.g., lower curve in Fig. 6D). The converse was generally true for extension-velocity sensitive neurones. This could suggest that the hysteresis is simply a long-lasting aftereffect of the phasic response. However a few phasic-tonic units did not follow this pattern. For example, the neurone represented in the upper curve of Fig. 7A responded phasically to flexions, but its tonic firing was higher at any given angle if that angle had been approached by an extension. Position-and-acceleration sensitive neurones also exhibited similar hysteresis to PV units, yet their phasic response was limited to 1-2 spikes per ramp. Some other neurones with mid-angle tonic maxima had response curves which crossed over near 60° (e.g., upper curve in Fig. 8D). In these cases the hysteresis meant that the firing frequency at any set angle was greater if that angle had been approached from a position further from the cross-over angle. Hofmann et al. (1985) noted phasic-tonic neurones in the stick insect which respond tonically as the leg was set to progressively more extended angles, but respond phasically to flexions. This information, and the differences in hysteresis mentioned above suggest that in many phasic-tonic neurones the tonic and phasic responses are largely uncoupled from each other. Mill & Lowe (1972) have also noted this phenomenon in the PD proprioceptor of decapods. Because the mechanism of sensory transduction in chordotonal scolopidia is still unclear it is difficult to speculate about the possible basis for such apparently contradictory responses. Some insight may be gained by recording from, and

staining individual receptors before fixing and sectioning the FCO for TEM examination. In this way it should be possible to clarify structural differences in the dendritic endings, scolopidia, and attachment cells of the various response types. As noted earlier, Field (in press) has begun this task by examining the fine structure of the FCO's main ligament. Further information about the mechanics of tension development in the various strands described by Field, and the degree of interaction between strands is now required. The strikingly non-linear responses of many tonic neurones to linear movements of the apodeme (especially the units with secondary maxima at 120° (Fig. 8)) highlight the need to clarify the interactions between mechanical and electrophysiological factors controlling FCO responses. Zill (1985a) made a brief study of the effect of injected current on the responses of various locust mtFCO neurones, but did not thoroughly characterise neurones before testing their response to current. In addition, he gave very little information about the details of each elicited response. A more thorough study of the relationships between injected current and firing frequency in characterised neurones would complement a morphological study, and would help explain the basis for range fractionation and hysteresis in these neurones.

Velocity effects on firing frequency

Although velocity thresholds were not systematically examined in the present study it is possible to make some generalisations about the responses of velocity-sensitive neurones. The range of angles and velocities tested in this work are similar to those used by the animal during walking: Burns (1973) reported that the metathoracic leg moved in the arc 40-90° at stepping frequencies of up to 9 Hz during straight walking. Assuming a sinusoidal protraction/retraction cycle, it is possible to determine that the maximal angular velocity during such movements will be approximately $1400°s^{-1}$. This is likely to be an upper limit because velocity is known to be controlled during both the stance and swing phase (Dean & Cruse 1986). For example, in the stick insect at least, a graph of leg position versus time tends to resemble a rounded saw-tooth pattern with constant velocity during both phases of the step. It is clear though, that the locust leg can be moved at higher velocities during kicking for example. At these higher velocities, proprioceptive feedback is likely to have a reduced influence because reflex latency will significantly interfere. In my work, velocities between 20 and $1000°s^{-1}$ were regularly used to test phasic responses of neurones in the range 0-120° of

leg angle. Some neurones were tested at lower velocities (5-20°s^{-1}).

Most velocity-sensitive neurones responded to all the velocities used (20-1000°s^{-1}), although different neurones could have different firing frequencies at a given velocity (compare Fig. 16A & B). Some neurones were markedly more sensitive to low velocities than most (e.g., Fig. 23A). No neurones saturated at higher velocities. Note however that the 2 upper velocities tested were usually 400 and 1000°s^{-1}: some neurones could have reached their peak firing frequencies at an intermediate value (e.g., 800 Hz). Zill (1985a) illustrated the responses of 2 phasic neurones to increasing frequencies of sinusoidal movement (from 0-9 Hz) over a 12° arc. Both these neurones were almost saturated at 5 Hz (corresponding to *maximal* velocities of 188°s^{-1}). However, in his text Zill stated that "at frequencies greater than 50-75 Hz these units show range saturation". If this is accurate, then the correct value for response saturation velocity would be 1880°s^{-1}, corresponding better with my information. Zill (1985a) also stated that below the saturation level, firing frequency increased linearly with increased frequency of stimulation. This does not agree with my results, nor with those of Hofmann et al. (1986): in these cases a log-log relationship was preferred. Mill & Lowe (1972) illustrated velocity sensitive neurones from the PD organ of *Cancer*, one of which had a linear increase in firing with increased velocity, and another which responded linearly to the log of velocity. Although Mill & Lowe give a regression coefficient (r = 0.966) for their log-linear curve, they do not give corresponding values for the same data plotted on other coordinate systems, so it is not possible to confirm that this is the best possible fit. Hofmann et al. (1985) did not fit regressions to their data. In my study, average r^2 values (i.e., mean values from the regressions for many neurones) were always higher for data plotted on log-log scales than for the same data plotted on log-linear or linear axes. However, individual neurones sometimes had higher r^2 values when they were plotted on linear or log-linear axes. Regression coefficients for all three possibilities were always high (worst fit: r^2 = 0.77). It is possible that different neurones have different stimulus-response relationships. Because I did not consistently test velocities at both extremes of sensitivity I am unable to compare the upper and lower thresholds of different neurones. This restricts any interpretation of the variation of shape or slope of the regression lines beacause each neurone may have been tested in a different parts of its sensitivity range.

Cohen (1963) illustrated another type of response for a flexion-sensitive neurone from the myochordotonal organ of *Cancer*: the slope of its response/

velocity curve increased at higher velocities in the range 0-16°s^{-1} (the opposite of saturation). It is not clear however if these velocities represent the full physiological range used by the animal, so this observation cannot be clearly compared to other studies.

Non-linearities in the stimulus-response behaviour of mechanoreceptive neurones may be introduced at a variety of steps in the pathway from mechanical input to firing frequency (Mann & Chapman 1975). These include viscoelastic properties of the surrounding tissue and/or cuticle, transients in the conductances of strain-sensitive channels in the transducer membrane, and accommodation of the axonal spike generating zone. Because the FCO is linked to the tibia by a relatively long connective tissue strand, it is likely that viscoelastic properties of the strand are very important in determining velocity sensitivity at least.

Viscoelasticity is a generalisation of viscosity and elasticity. The ideal linear elastic element is a spring, while the ideal viscous element is a dashpot (e.g., a loose-fitting piston in an oil-filled cylinder arranged so that oil can flow back past the piston as it moves). In a viscoelastic model the two elements can be arranged in series or in parallel, or in combinations (with more than one element of each) (Bland 1960). Modeling such systems requires determination of at least 2 parameters: the modulus of the spring element(s), and the viscosity of the dashpot(s). I am not aware of any attempts to measure or estimate the relevant parameters of connective tissue strands, although considerably work has been undertaken on the viscoelastic properties of red blood cell membranes. Without this knowledge it is not practical to speculate on the expected contribution of viscoelasticity to the shape of the stimulus response curves presented here. It should be possible to simply measure the velocity and displacement of the FCO main ligament close to the FCO neurones during physiological movements of the tibia. This direct approach may be the most useful way to investigate the component mechanisms underlying range fractionation and hysteresis.

CHAPTER FOUR

Locust metathoracic femoral chordotonal organ neurones have central projection patterns which correspond to their response

ABSTRACT

The locust metathoracic femoral chordotonal organ contains neurones which respond to tibial position, velocity, or acceleration, or to combinations of these parameters. Discriminant analyses confirmed that neurones with different responses to tibial movements had different central branching patterns. Some aspects of the projections were consistent for all neurones (e.g., the path taken by the main neurite through the metathoracic ganglion), whereas other regions of branches were reduced or missing in some response classes. Some position-and-acceleration receptors had no main branches off the main neurite, and must therefore make relatively restricted contact with motorneurones and interneurones. Phasic or tonic neurones which responded in ranges of tibial extension had branches which projected further medial in Dorsal Commissures III and IV. This type of mapping does not appear to have been described previously. I compare my results with previous studies of tonotopic and somatotopic mapping in the insect CNS.

INTRODUCTION

The central projection patterns of many insect sensory receptor types have been studied in great detail (cricket clavate hairs: Murphey et al. 1980; cricket bristle hairs: Johnson and Murphey 1985; orthopteran tympanal afferents: Römer et al. 1988, Oldfield 1983; locust hairplates and campaniform sensilla: Bräunig et al. 1981, 1983, Hustert et al. 1981, Pflüger et al. 1981; locust vibration receptors: Grosch et al. 1985, Römer 1985; locust chordotonal organs: Bräunig et al. 1981, Burrows 1987, Field and Pflüger 1989, Hustert 1978. See Pflüger et al. 1988 for other references). While some of these studies contain attempts to fill and categorise individual receptors (e.g., Murphey et al. 1980, Johnston and Murphey 1985, Römer et al. 1988), others (including those of chordotonal organs) have relied on whole-nerve axonal diffusion of dye to stain the entire population of afferents. This does not allow accurate investigation of the variation between

afferents, and precludes attempts to correlate structure with response.

The central branching patterns of physiologically similar afferent neurones often show variations in form. Mapping occurs if these variations relate in a systematic way to aspects of the neurones' responses. Two types of mapping have been described from the insect CNS: (1) tonotopic mapping is the orderly arrangement of afferent neurones according to their sensitivity to different frequencies of vibration. For example, the neurones which make up the ordered arrays of cell bodies in cricket tympanal organs (crista acoustica) project into the anterior intermediate sensory neuropile of the CNS in an pattern related to their physiological response (Oldfield 1983, Römer et al. 1988). In contrast, the 4 groups of receptor cells from the locust ear project to 4 different areas of this same neuropile, but there is no discernible mapping within each area (Römer et al. 1988); (2) somatotopic (topographic) mapping is the arrangement of afferents according to the position of their peripheral somata. For example, the terminal projections of identifiable cricket clavate hairs located on the cerci are unique and reproducible from specimen to specimen (Murphey et al. 1980). The branching pattern of a clavate hair neurone is correlated to the cell's 'birthday' (moult in which it first appears) and its position on the cercus.

The femoral chordotonal organ (FCO) monitors movements of the tibia relative to the femur. It contains neurones which respond to tibial position, velocity, or acceleration, or to combinations of these parameters (Matheson 1990). By using whole-nerve backfilling techniques, Field and Pflüger (1989) have shown that the functions and central projections of the two scoloparia (groups of neurones) present in the locust pro- and mesothoracic FCO's are quite different from each other. Those authors did not, however, attempt to stain or characterise individual neurones. Likewise, Burrows (1987) shows metathoracic FCO (mtFCO) projections derived only from whole-nerve fills.

In this paper I present for the first time a detailed study of the central projections of individual, physiologically characterised neurones from a joint chordotonal organ, the locust mtFCO. Cluster and discriminant analyses are used to help establish relationships between response class and central projection pattern.

MATERIALS AND METHODS

Adult locusts (*Locusta migratoria*) from our laboratory culture were used for all experiments. The results are based on 101 successful recordings from 119

animals.

The terminology, and the stimulating, recording, and dye injection techniques used in this paper have been described previously (Matheson 1990), with some modifications outlined in the brief summary below.

Animals were restrained ventral side up in plasticene. Movements of the mtFCO apodeme at controlled velocities mimicked tibial movements between 0 and 120°. The flexor strand was held motionless at 60°. A window cut in the ventral thoracic cuticle gave access to the metathoracic ganglion, which was supported on a wax-coated silver platform. A silver loop slightly larger in diameter than the ganglion was lowered over the ganglion to minimise movements, and to hold the large longitudinal tracheae away from the recording site in n5. Care was taken not to crush the connectives or other nerves entering the ganglion. The tracheal supply to the ganglion was left intact, but some air-sacs associated with the thoracic musculature were removed. A groove cut in a third silver platform, positioned under n5 close to the ganglion, provided great stability for intracellular recording. Drops of locust saline (pH 6.8) were added if required to prevent drying out of the preparation.

After characterisation and dye-filling of a mtFCO afferent neurone (hexamminecobalt (III) chloride, 30 min, 1.5 Hz, 200 ms pulses, 2.5 V) the electrode and support platforms were removed, and the dye allowed to diffuse for a further 30 min. The metathoracic and mesothoracic ganglia were then dissected out together, and the cobalt visualised using standard procedures, including silver intensification. Neurones were drawn with the aid of a camera lucida, and photographed at several (4-8) planes of focus. Preparations in which more than one cell stained have not been included in the analysis, with the following exception: if one cell in a multiple fill was much darker than the others it was assumed that this was the characterised cell, while the others had merely taken up traces of dye during short periods of recording from them earlier in the experiment. This possible ambiguity has been noted in the results where applicable. Poorly stained cells are included in the analysis, but are noted as such.

Selected ganglia were embedded in Paraplast wax for serial sectioning at 7 *u*m. Sections were counterstained in 0.1% Toluidine Blue and mounted in Eukitt. Cobalt filled branches were drawn in relation to recognisable tracts and neuropiles visible using interference contrast optics.

Cluster analysis

The purpose of cluster analysis is to place objects (in this case neurones) into groups or clusters suggested by the data (branching pattern), not defined *a priori*, such that objects in a given cluster tend to be similar to each other in some sense while objects in different clusters tend to be dissimilar.

In order to objectively categorise the branching patterns of mtFCO neurones a cluster analysis was carried out (SAS: standardised variables, average linkage method. SAS Institute Inc.). The variables used for this analysis were derived from neurones drawn in wholemount from the ventral aspect. They were:

1. The presence of a branch in the anterior medial bundle;
2. The presence of a branch in the posterior medial bundle;
3. The presence of branches in both medial bundles;
4. The presence of primary dorsal lateral branch(es);
5. The presence of prominent ventral branch(es) anterior to the main neurite;
6. The distance from the midline to the termination of the branch in the anterior bundle;
7. The distance from midline to the termination of the branch in the posterior bundle;
8. The length of the longest dorsal lateral branch;
9. The area covered by branches; and
10-33. The density of branches in each of 24 grid squares (see below for further details).

Distances were standardised as a percentage of the length of the ganglion, while the area was expressed in arbitrary units divided by the square of this length. This was considered necessary to counter variation in ganglion size related to different sized individuals, and caused by possible shrinkage during tissue processing. Where there was no branch in the anterior or posterior bundles, the distance given (at variable 6 or 7) was arbitrarily set to 25 (for anterior) or 35 (posterior) - the approximate average standardised distance from the midline to the main neurite at the two levels.

The grid used for the last 24 variables was based on neurone morphology, rather than on landmarks visible on the surface of the ganglion (which are known to vary considerably between individuals (Burrows and Hoyle 1973)). It is apparent from the whole-organ locust FCO projections of Field and Pflüger (1989) (msFCO distal scoloparium) and Pflüger et al. (1988) (mtFCO) that the

most reliable features of filled FCO neurones are: (1) the location of the first main lateral branch (usually an anterior dorsal branch); and (2) the points where the 2 prominent medial branches leave the main neurite. I used the branch-point of the first main lateral branch as the starting location of the grid (* in Fig. 1). From this point a straight line (A-B in Fig. 1) was projected along the axis of the main neurite (which was usually quite straight). A second (temporary) line (C-D in Fig. 1) was drawn along the anterior medial branch (where present) to intersect with the first line. The point of intersection of these 2 lines (** in

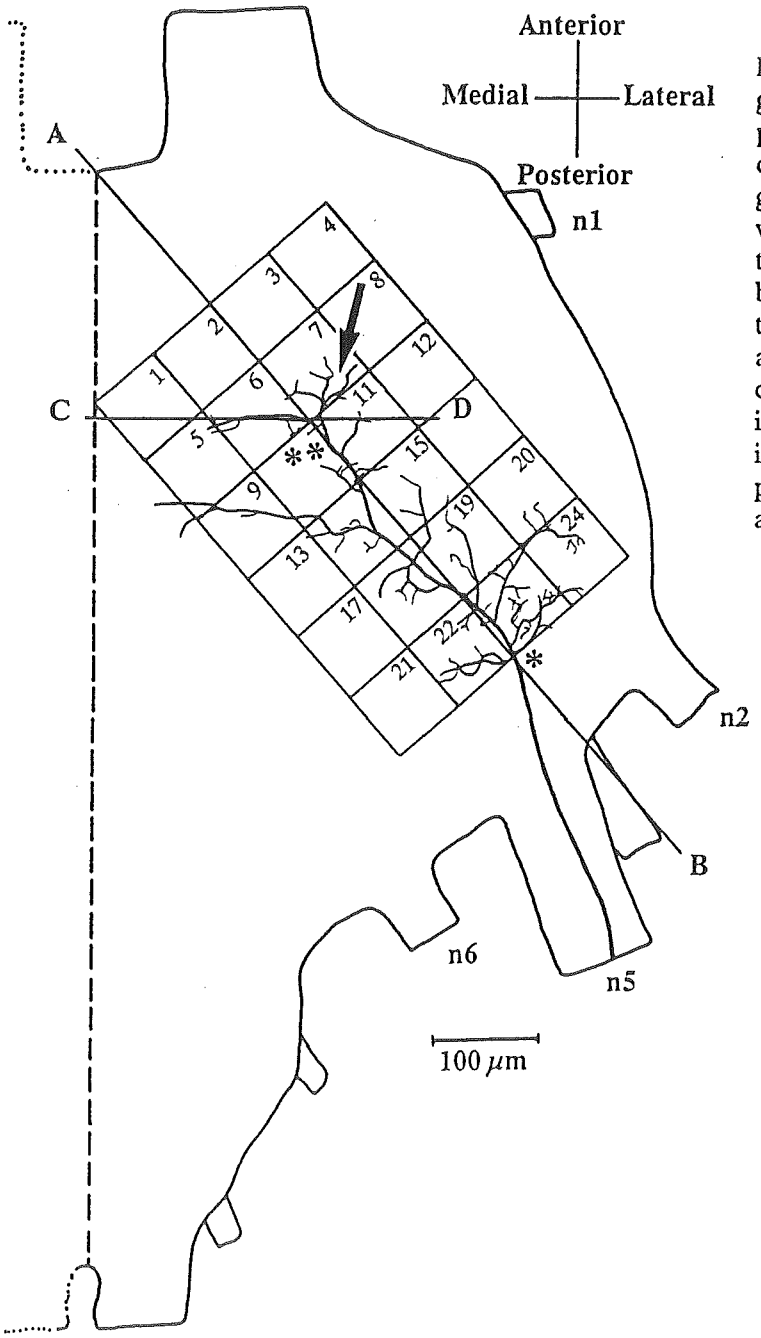


Figure 1. Locust metathoracic ganglion containing the central projections of a single stained chordotonal organ neurone. The ganglion is viewed from its ventral aspect, with anterior at the top. The midline is indicated by the vertical dashed line to the left. The main leg nerves are numbered (n1-n6). The construction of the overlying grid is described in the text. All other illustrations of ganglia in this paper are in the same orientation as Fig. 1.

Fig. 1) was used as the second fixed point. This second point usually corresponded well with the location at which the anterior medial branch left the main neurite. The distance between the points was divided by 4 to give a convenient mesh size, and a grid of 24 squares was projected over the ganglion (Fig. 1). Three neurones did not possess 'main' lateral branches: in these cases I used the first visible lateral branch. Some neurones did not posses branches which clearly ran in the anterior medial bundle. In these cases it was possible to approximate the second fixed point by using the location where one or more fine branches (arrow in Fig. 1) left the main neurite to travel anteriorly and laterally for a short distance. The main neurite generally ended at this point. Grid squares were numbered from 1 to 24 as shown in Fig. 1.

The density of branches in each grid square was scored on a 3 point scale: 0 = none; 1 = a few; 2 = branches throughout square.

Discriminant Analysis

Discriminant analysis is a group of procedures with the general function of revealing relationships between observations (in this case, neurones) placed in *a priori* groups (here based on physiological response classes as defined in Chapters Two and Three) and a range of measured quantitative variables (aspects of morphology).

I have used discriminant analyses to: (a) indicate neurones with branching patterns markedly different to others in the same response class; (b) test which morphological variables are the best predictors of response class, and (c) provide a graphical representation of the grouping of the response classes. The SAS procedures (SAS Institute Inc.) used for each of these were respectively: (a) Proc discrim (discriminant function analysis based on the pooled covariance matrix); (b) Proc Stepdisc (stepwise discriminant analysis); and (c) Proc Candisc (canonical discriminant analysis). These procedures were each run twice: once with the dataset divided into 9 main response classes (see below); and again with the same dataset subdivided into a total of 30 subclasses (distinguishing between, for example, V^+ neurones which fired only close to 0° and those which fired across the full range of leg angles).

The following 9 response classes were used initially. Subclasses and their index numbers are indicated in brackets after each main class. See Matheson (1990) for details of response classes and terminology. Note that classes are numbered from 1-9, and subclasses are classified from 0-9, and A-T. The fact

that some classes and subclasses have the same index number (e.g., class 1, subclass 1) does not imply anything about their responses or morphology.

1. Neurone responded to accelerations only (included $?A^{+}$ (0), $?A^{+-}$ (1), $?A^{-}$ (2), and ?AS (3) responses);
2. Neurone responded to position and acceleration (included $P^{0}?A^{x}$ (4) and $P^{mid}?A^{x}$ (5) responses);
3. Neurone responded to position and flexion velocity (included $P^{0}V^{+}$ (6), $P^{0}V^{+wide}$ (7), $P^{0}V^{+0}$ (8), and $P^{mid}V^{+}$ (9) responses);
4. Neurone responded to position, flexion velocity, and positive acceleration (included $P^{0}V^{+}?A^{+}$ (A) and $P^{mid}V^{+}?A^{+}$ (B) responses, and 1 neurone with a $P^{20}V^{+}?A^{-}$ (C) response);
5. Neurone responded to position and extension velocity (included $P^{mid}V^{-}$ (E) and $P^{120}V^{-}$ (F) responses, and 1 neurone with a $P^{0}V^{-}$ (D) response);
6. Neurone responded to position, extension velocity, and negative acceleration (included $P^{0}V^{-}?A^{-}$ (G), $P^{mid}V^{-}?A^{-}$ (H), and $P^{120}V^{-}?A^{-}$ (I) responses);
7. Neurone responded to flexion velocity only (included V^{+} (J), V^{+wide} (K), and V^{+0} (L) responses, and 2 neurones with a $V^{+}?A^{+}$ (M) response);
8. Neurone responded to extension velocity only (included V^{-} (N), V^{-mid} (O), V^{-wide} (P), and V^{-120} (Q) responses);
9. Neurone responded to extension velocity and negative acceleration (included $V^{-}?A^{-}$ (R), $V^{-mid}?A^{-}$ (S), and $V^{-wide}?A^{-}$ (T) responses).

RESULTS

Background

Recordings made from the axons of mtFCO neurones near the metathoracic ganglion (this paper) were generally more stable than recordings made from the same population of axons distally in the leg (Matheson 1990). Cobalt fills of mtFCO neurones were generally of good quality (i.e., only a single axon stained, and good filling of fine branches), although a few appeared unusually 'blebby'. In order to lay a groundwork for describing the projections of individual receptors I will first summarise the overall projection pattern described from whole-nerve cobalt backfills of the mtFCO by Burrows (1987) and Pflüger et al. (1988). The neurone illustrated in Fig. 1 (present paper) has branches in all regions occupied by neurones in whole-nerve fills, and therefore provides a useful reference for overall morphology.

After entering the ganglion via N5 the FCO axons immediately give off branches (termed dorsal-lateral branches in my work) which run anteriorly and dorsally into the anterior and posterior lateral association centres (aLAC and pLAC). The axons continue to run anterior-medially in a root of N5 (probably 5ii or 5iii) giving off branches along the way. Two parallel bundles of collaterals extending to within 50 *u*m of the midline are prominent and consistent (I term these bundles the anterior and posterior medial bundles): the anterior-most passes between the ventral intermediate (VIT) and ventral lateral (VLT) tracts before joining into dorsal commissure III (DCIII) where it ends. The posterior bundle passes between VIT and the dorsal intermediate and dorsal medial (DIT, DMT) tracts before entering DCIV and terminating approximately 50 *u*m short of the midline. Most of the remaining branches lie at the level of VIT and the median ventral tract (MVT). No branches are found in the ventralmost or lateral ventral association centres (vVAC, lVAC). Burrows (1987) illustrated some fibres quite ventral in the ganglion (his Fig. 3B), but these do not agree with the mtFCO projections drawn by Pflüger et al. (1988), and almost certainly represent axons of leg hair afferents. Burrows (1987) also illustrated the central projections of some individual mtFCO neurones derived from the whole-nerve stains. These will be discussed later.

The first discriminant analysis (SAS Proc Discrim) was used to identify misclassified observations (i.e., neurones with branching patterns significantly different from others in the same physiological response class). The SAS procedure identifies misclassified observations by calculating (on the basis of morphological similarity) the probability that a given neurone would be classified into each possible class. Neurones which have been put by the investigator into a class other than that with the highest probability are termed misclassified. These results were used as a guide for reassessing individual neurone's classifications, and as a check of the accuracy of the dataset before running the canonical discriminant analyses described below. Neurones which the SAS procedure indicated were misclassified were only reclassified if a re-examination of their original response data revealed an error of interpretation (i.e., neurones were not reclassified solely on the basis of the discriminant analysis). Of 20 neurones indicated as misclassified, only 1 was found to be clearly in error, and was reclassified. The outcome of the initial discriminant analysis is not formally presented as Results.

Central projections of mtFCO neurones

Different neurones clearly had different patterns of central morphology. However the axon of all neurones ran as far anteriorly as the base of DCIII (the point where the anterior medial bundle turns medially and dorsally to enter the commissure). Neurones which had a branch in the posterior medial bundle always had a branch in the anterior medial bundle. Some neurones had anterior but not posterior medial branches.

The canonical discriminant analysis based on the 9 main response classes produced 8 canonical functions to describe variation in the morphological data. The first 2 functions were able to explain significant amounts of this variation ($P < 0.0005$ for both), while the third was only significant at the 1% level ($P = 0.095$). Therefore the response classes were plotted against the first 2 canonical variables, to yield the groupings shown in Fig. 2. The analysis also indicates the order of importance of morphological variables in providing separation along each axis of Fig. 2. In this case, the four most important variables are the same for both plotted axes (length of posterior medial branch > presence of posterior medial branch > density of branches in grid 5 > length of anterior medial branch). On the first axis, the next (fifth) most important variable is the overall area covered by branches, while on the second axis, the fifth-most important variable is the density of branches in grid 23.

The final groupings can be interpreted in terms of both morphology and response. All the neurones in classes 5,6, and 9 (to the right of Fig. 2) have both anterior and posterior medial branches, and include a V^- component (extension sensitivity) in their response. These groups include a total of 29 neurones. Neurones in class 8 also have V^- responses, and some can be seen almost as far right as classes 5,6, and 9 in Fig. 2). Because the lengths of the medial branches are the most prominent morphological features of many neurones (and are an important criterion for separation along the canonical axes) it is convenient to divide the results section on this basis also. I will therefore concentrate on the right-most groups of Fig. 2 in the next section, before going on to examine the classes which fall further to the left.

Neurones with 2 well-developed medial branches

The general form of the branching pattern of neurones in these right-most groups is illustrated in Fig. 3A (a $V^-?A^-$ neurone). Sections (Fig. 3Bi-v) were taken through this preparation at locations A-E indicated on Fig. 3A. In this and

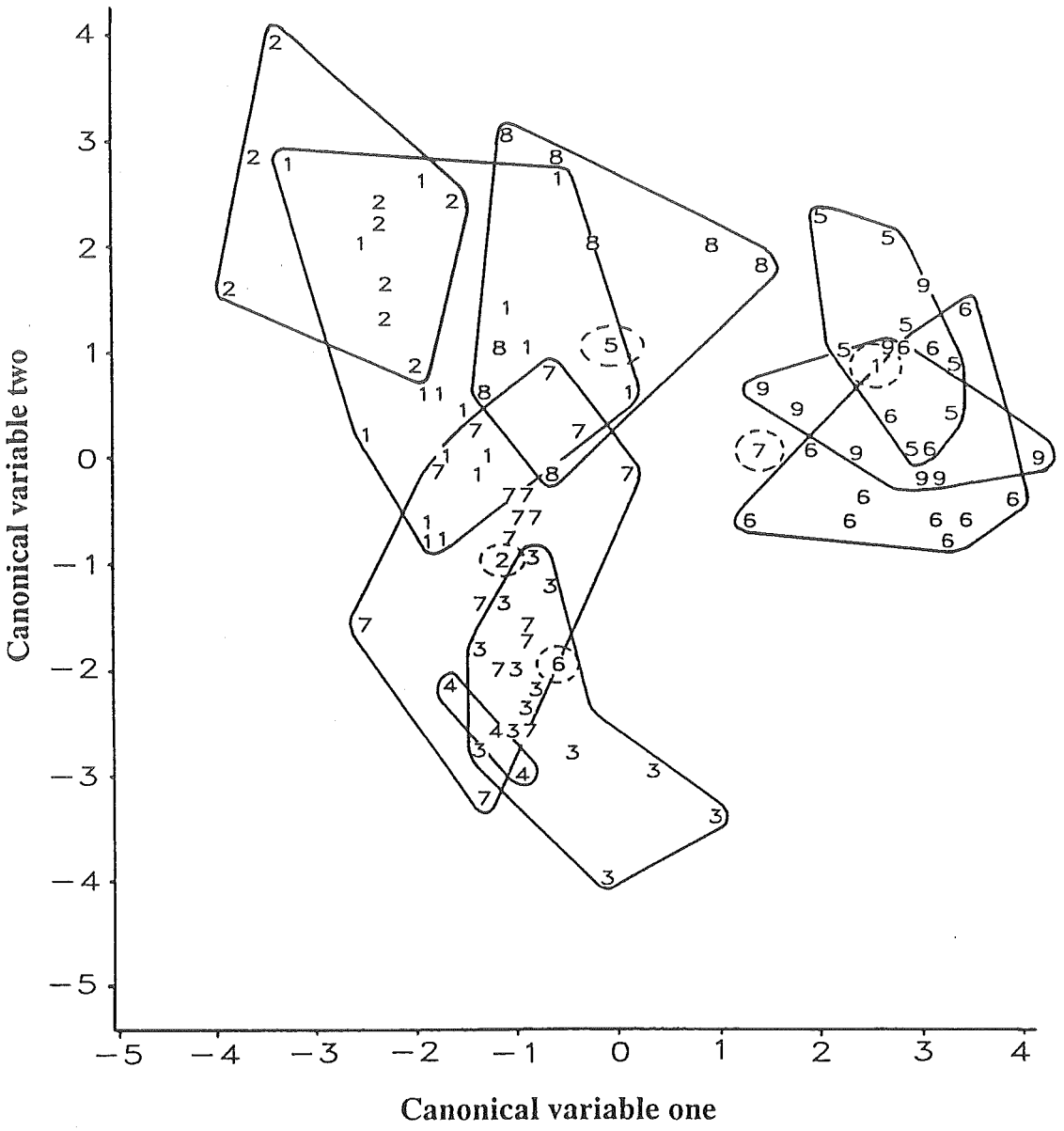


Figure 2. Plot of response classes against the first two canonical variables. Solid lines have been drawn around the boundaries of each response class. Five neurones which fell well away from others in their class are indicated by dashed circles, and are mentioned in the text.

following figures of sections, the main neurite is indicated with an asterisk if it is clearly identifiable. The anteriormost branches were found at the level of, and lateral to VIT (Fig. 3Bi). The anterior medial branch in DCIII (Fig. 3Bii) terminated in a small cluster of fine twigs, some of which plunged ventrally, medial to VIT (Fig. 3Biii). The prominent somata and neurite of FETi can be seen in this section. A section cut at location C of Fig. 3A (Fig. 3Biv) revealed the posterior medial branch running in DCIV to end near DMT. Dorsal lateral branches reached nearly to the level of DIT, while some ventral branches lay

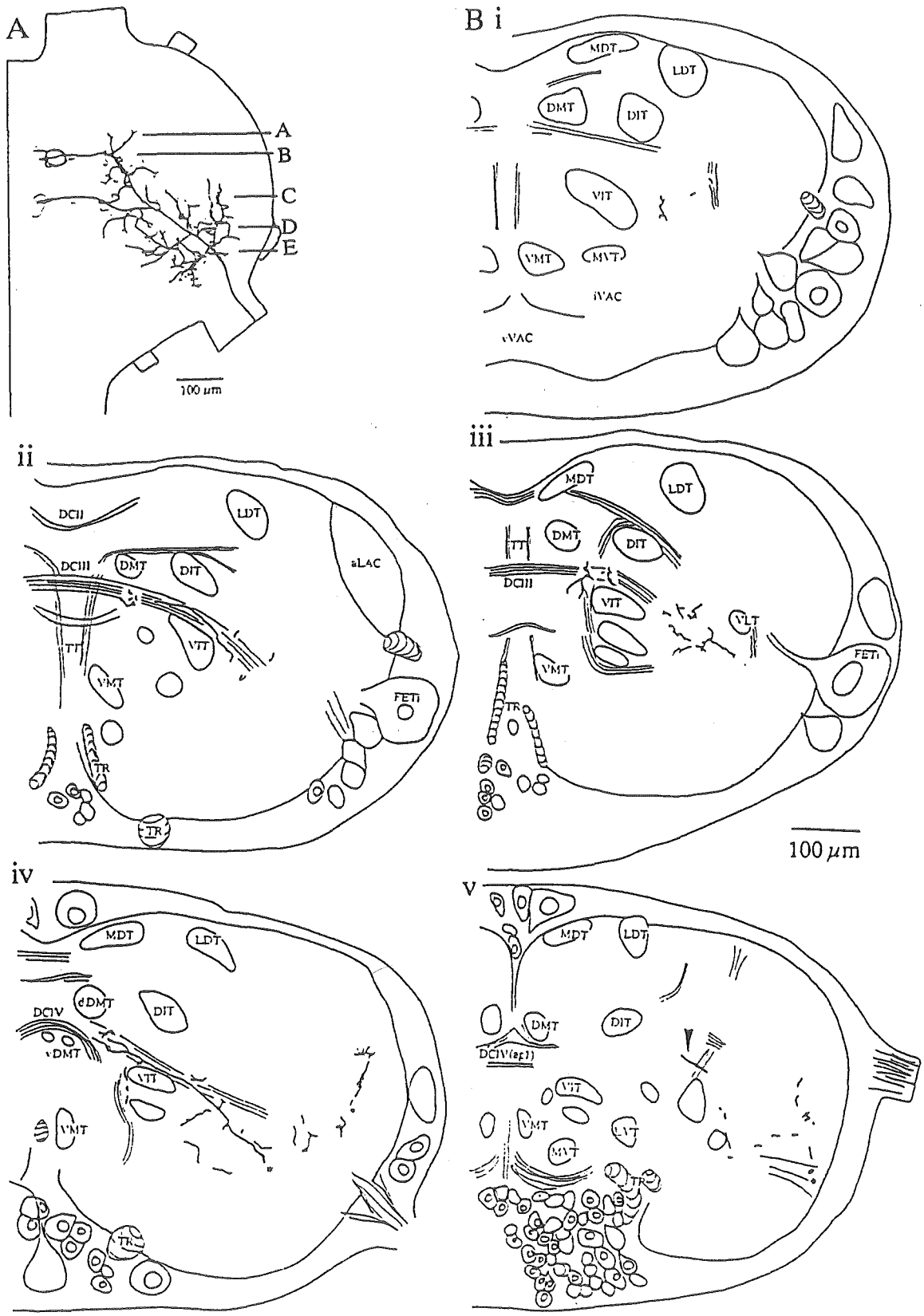


Figure 3A, Bi-v. (A) Central projections of a V⁻?A⁻ (class 9) neurone. The exit point of the medial trachea from the ganglion is shown as a solid circle overlapping with the branches in the anterior medial bundle. Sections were taken at locations A-E. (Bi-v) Transverse sections through the ganglion illustrated in Fig. 3A. Each section is mentioned in detail in the text. Abbreviations are explained in the text (except TR = trachea), and follow Pflüger et al. (1988).

below VMT. A section (Fig. 3Bv) cut as the main neurite reaches the edge of the neuropile contained further dorsal lateral branches, and one dorsal branch further medial (arrow).

Only 5 neurones which had a V^- component did not have branches in both medial bundles. Of these, 3 were poorly filled (2 from class 8, 1 from class 6 - see below). The remaining 2 were both wide-range V^- units (class 8), 1 of which was unusually slow to stop firing after a stimulus had ceased. The poorly filled neurone from class 6 was positioned lower and further left in Fig. 2 than others in its class (dotted outline). This reflects its apparent lack of medial branches, and the general sparseness of its other branches, apparently artifacts caused by to poor dye filling.

A second canonical discriminant analysis was carried out on 30 subclasses of response (Fig. 4A). It was able to clarify more subtle differences between response classes because variation within subclasses was less than that within the 9 main classes. The first axis of this analysis was significant at $P < 0.0005$ level, while the second axis was only significant at $P < 0.1$ level. The order of importance of the first 5 morphological variables for placing neurones along the two plotted canonical axes are respectively: (axis 1: length of posterior medial branch > presence of posterior medial branch > density of branching in grid 5 > area > density of branches in grid 12; axis 2: presence of posterior medial branch > length of posterior medial branch > length of anterior medial branch > presence of anterior medial branch > area). Figures 4B-E are simply subsets of the groupings shown in Fig. 4A, and are referred to in the following descriptions for the sake of clarity. Individual observations have been removed from these figures, and the subclass names and responses added. Figure 4A, on the other hand provides an overview of all the clusters.

Classes 5,6, and 9 (Fig. 2) include subclasses E-I and R,S,T (Fig. 4B). For the purposes of this more detailed description it is useful to include subclasses of class 8 (so that all neurones with a V^- component are considered together): these are subclasses N-Q. No neurones in subclasses D,G, or Q were successfully stained (although several have been recorded from in these and other experiments), thus these classes are missing from Fig. 4B.

Pure extension-velocity sensitive units. Pure velocity units fell into 3 groups related to their subclasses (O, N, P). As suggested by their location to the right of Fig. 4B, neurones in subclass O (V^{-mid}) had branches in both medial bundles (Fig. 5). Neurones in subclass N (V^-) had relatively sparse branching (Fig. 6). One of

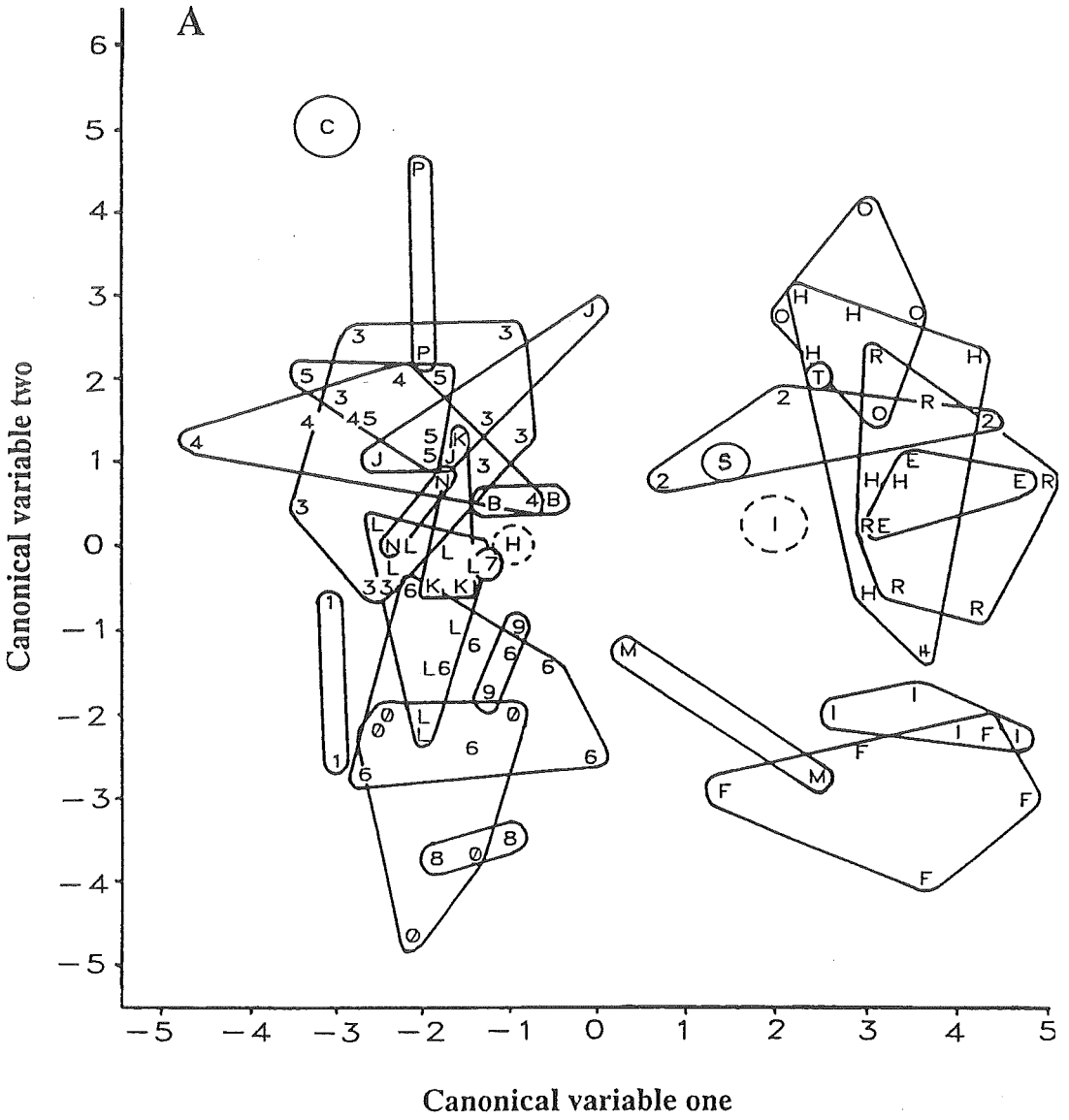


Figure 4A. Plot of response subclasses against the first 2 canonical variables. The location of individual observations within subclasses are shown. A break-down of this figure is presented in Fig. 4B-E overpage.

these had a branch in the anterior medial bundle, but the other (which was poorly filled) did not. Two neurones with V^{-wide} responses (subclass P) were stained (1 poorly). Both had a short branch in each medial bundle, and sparse branching elsewhere (Fig. 7).

Position-and-extension-velocity sensitive neurones. Neurones sensitive to position and extension velocity ($P^{mid}V^-$ (subclass E), and $P^{120}V^-$ (subclass F)) fall into 2 groups to the right of Fig. 4B (no P^0V^- (subclass D) neurones were successfully stained). $P^{mid}V^-$ neurones (E) lie higher than $P^{120}V^-$ (F) neurones. The only

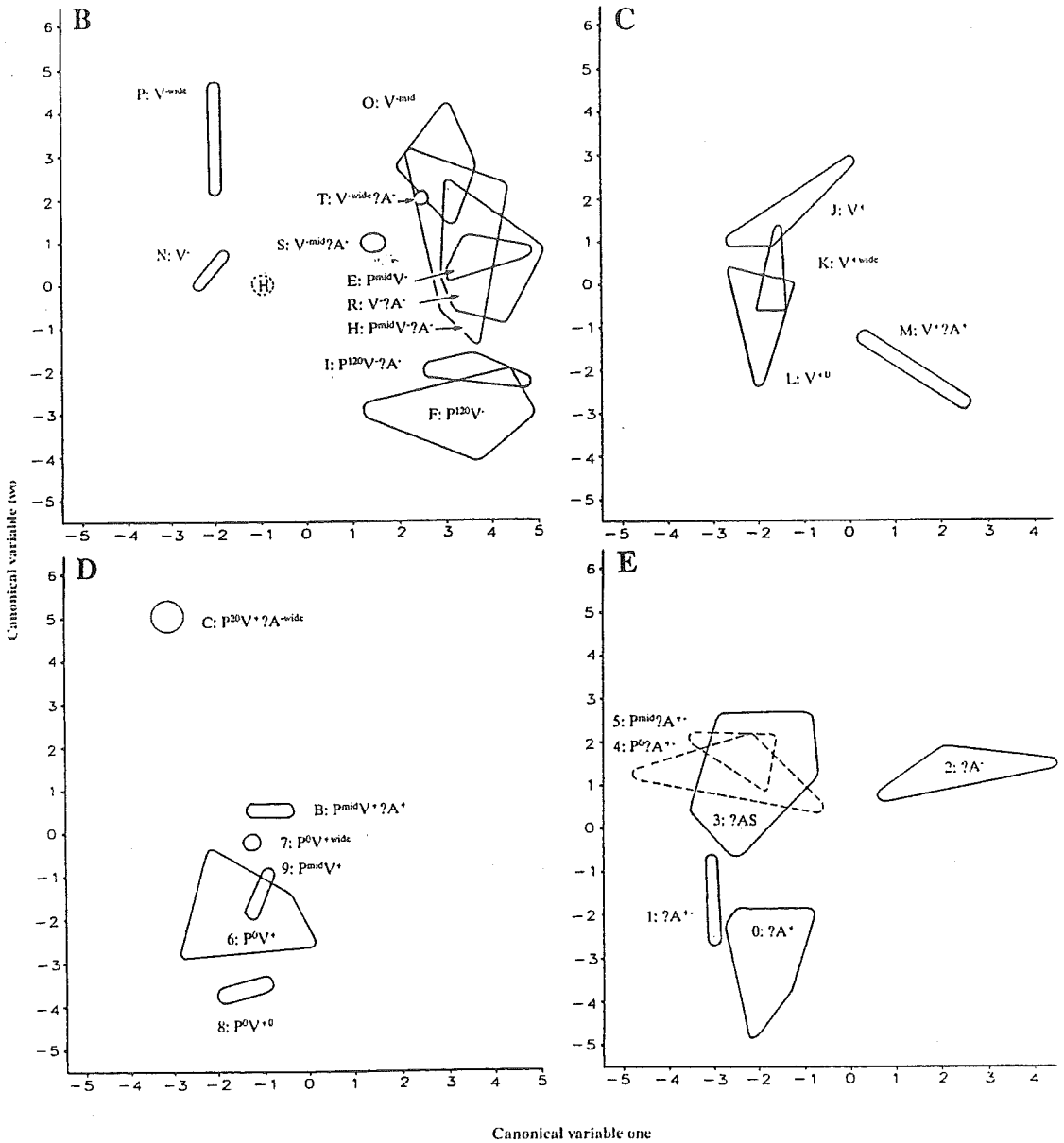
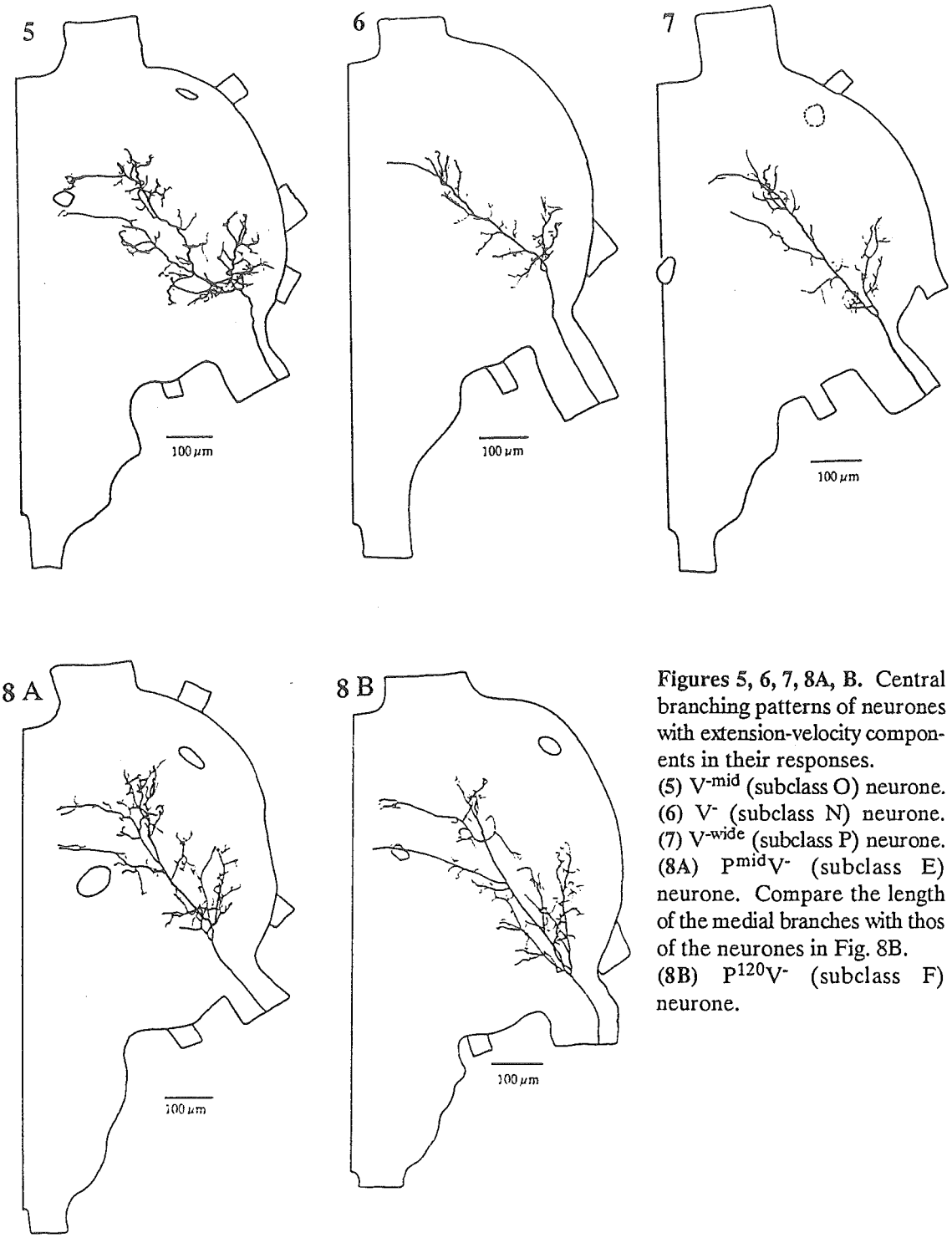


Figure 4B-E. Subdivision of the groupings shown in Fig. 3A. **(B)** Outlines of position-and-extension-velocity sensitive, and extension-velocity-and-acceleration subclasses. **(C)** Outlines of flexion-velocity, and flexion-velocity-and-acceleration subclasses. **(D)** Outlines of position-and-flexion-velocity subclasses. **(E)** Outlines of acceleration, and position-and-acceleration subclasses. The classification of the subclasses bounded by dashed lines is discussed in the text.

subjective difference in their branching seems to be the length of the longest dorsal lateral branch (i.e., how far anterior it reaches). Neurones with maximal tonic sensitivity near 120° generally had longer branches in the medial bundles than did mid-position sensitive neurones (compare Figures 8A & B which illustrate the branching of representative neurones from the $P^{mid}V^-$ and $P^{120}V^-$ classes



Figures 5, 6, 7, 8A, B. Central branching patterns of neurones with extension-velocity components in their responses.
(5) V^{-mid} (subclass O) neurone.
(6) V^{-} (subclass N) neurone.
(7) V^{-wide} (subclass P) neurone.
(8A) $P^{mid}V^{-}$ (subclass E) neurone. Compare the length of the medial branches with thos of the neurones in Fig. 8B.
(8B) $P^{120}V^{-}$ (subclass F) neurone.

respectively. Because of the small sample sizes this difference could not be tested.

Position,-extension-velocity,-and-acceleration sensitive neurones. Neurones sensitive to position, extension-velocity, and acceleration ($P^{mid}V^{-}?A^{-}$ (subclass H), and $P^{120}V^{-}?A^{-}$ (subclass I) also form 2 groups in Fig. 4B: no $P^{0}V^{-}?A^{-}$ (subclass G)

neurones were stained. The distribution pattern is similar to that for position-and-velocity receptors, with mid-range units (subclass H) plotted higher on the Y axis than corresponding flexion-range (I) units. The loci of $P^{mid}V^-$ and $P^{mid}V^-?A^-$ neurones overlap, as do the loci of $P^{120}V^-$ and $P^{120}V^-?A^-$ neurones. The morphology of position-velocity-and-acceleration units is very similar to that of position-and-velocity units (Fig. 8A,B), and is therefore not illustrated separately. Neurones in this group with tonic maxima at 120° had longer anterior medial branches than those with maximal tonic firing at mid-angles (t test: $P < 0.03$). Their posterior medial branches were also longer, but not significantly ($P = 0.16$). There was no significant difference in the lengths of the longest dorsal lateral branch ($P = 0.23$), although on average this branch was longer for mid-position units.

Extension-velocity-and-acceleration sensitive neurones. The groupings of extension-velocity-and-acceleration sensitive units ($V^-?A^-$ (subclass R), $V^{-mid}?A^-$ (S), and $V^{-wide}?A^-$ (T)) did not overlap on the canonical discriminant plot (Fig. 4B), but their branching patterns appeared very similar. They had the same general morphology as the position-and-velocity neurones described above (note the large extent of overlap between subclasses R ($V^-?A^-$), T ($V^{-wide}?A^-$) and H ($P^{mid}V^-?A^-$), and E ($P^{mid}V^-$) in Fig. 4B).

Relationship between phasic and tonic position sensitivity and branching pattern. In order to investigate further the relationships between the length of various branches and positional sensitivity, all neurones with branches in both the anterior and posterior medial bundles which had clear position-dependent maxima in either their tonic or phasic components were pooled. The standardised length of the longest dorsal lateral branch, and the standardised distance from the midline of the ganglion to the termination of each medial branch were regressed against the leg angle giving maximal response (Fig. 9A-C). The slopes of the regressions for both medial branches (Fig. 9A,B) are significantly different from zero (anterior: $P < 0.05$, posterior: $P < 0.0001$), indicating that these branches tend to terminate closer to the midline in extension-velocity sensitive neurones with phasic or tonic firing maxima near 120°. There is considerable variation about both lines (r^2 values are 0.14 and 0.56 respectively). The slope of the regression for length of dorsal lateral branches (Fig. 9C) was not significantly different from zero ($P = 0.25$).

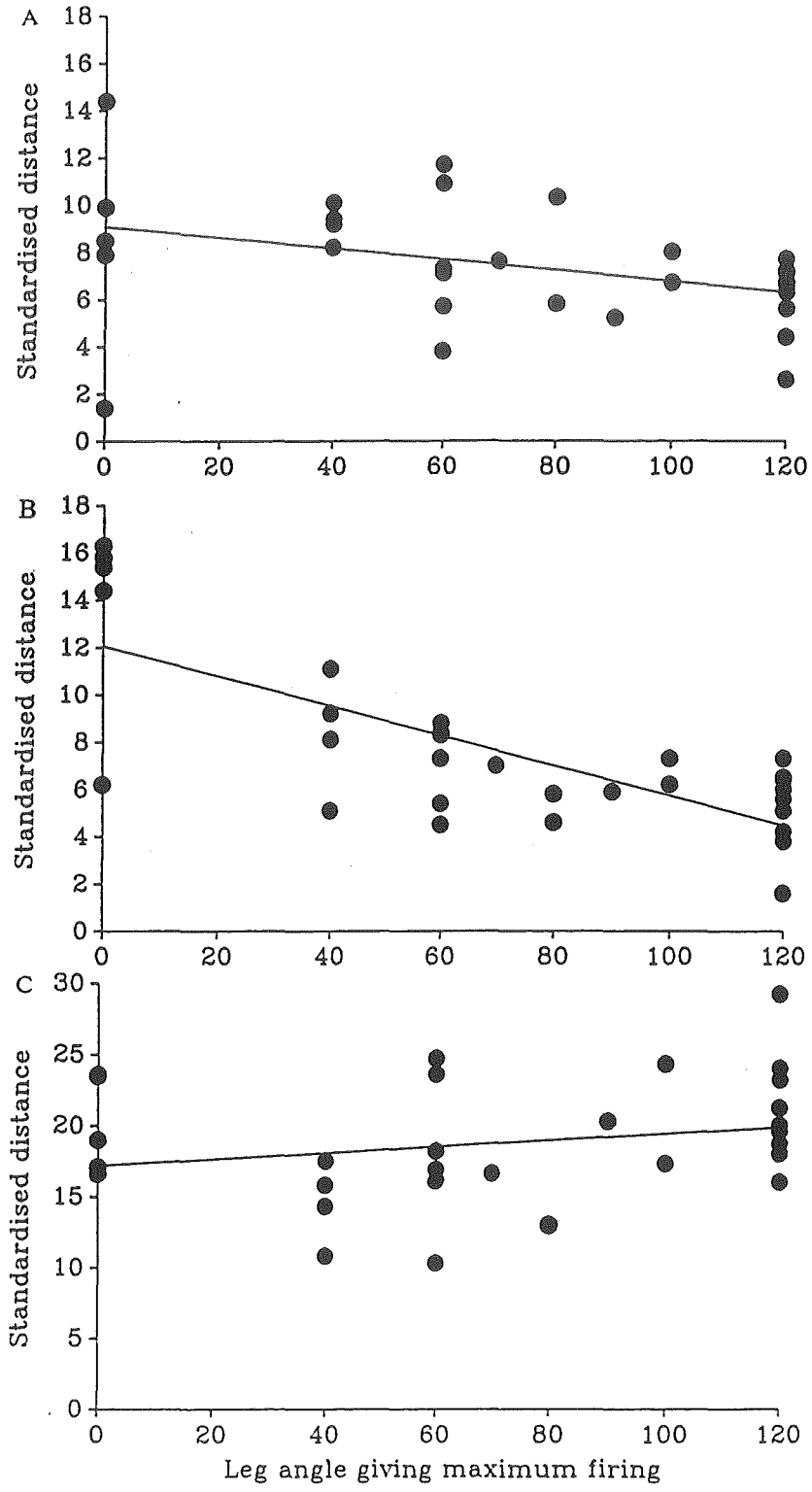


Figure 9A-C. Linear regressions of medial and dorsal branch lengths against an aspect of physiological response: the leg angle giving maximal phasic or tonic firing. All neurones with a branch in both medial bundles were pooled. (A) Length of anterior medial branch ($Y = -0.023X + 9.1$. $r^2 = 0.14$). (B) Length of posterior medial branch ($Y = -0.063 + 12.1$. $r^2 = 0.56$). (C) Length of dorsal lateral branch ($Y = 0.022X + 17.1$. $r^2 = 0.22$).

Neurones with a short (or no) branch in the posterior medial bundle

Flexion-velocity sensitive neurones

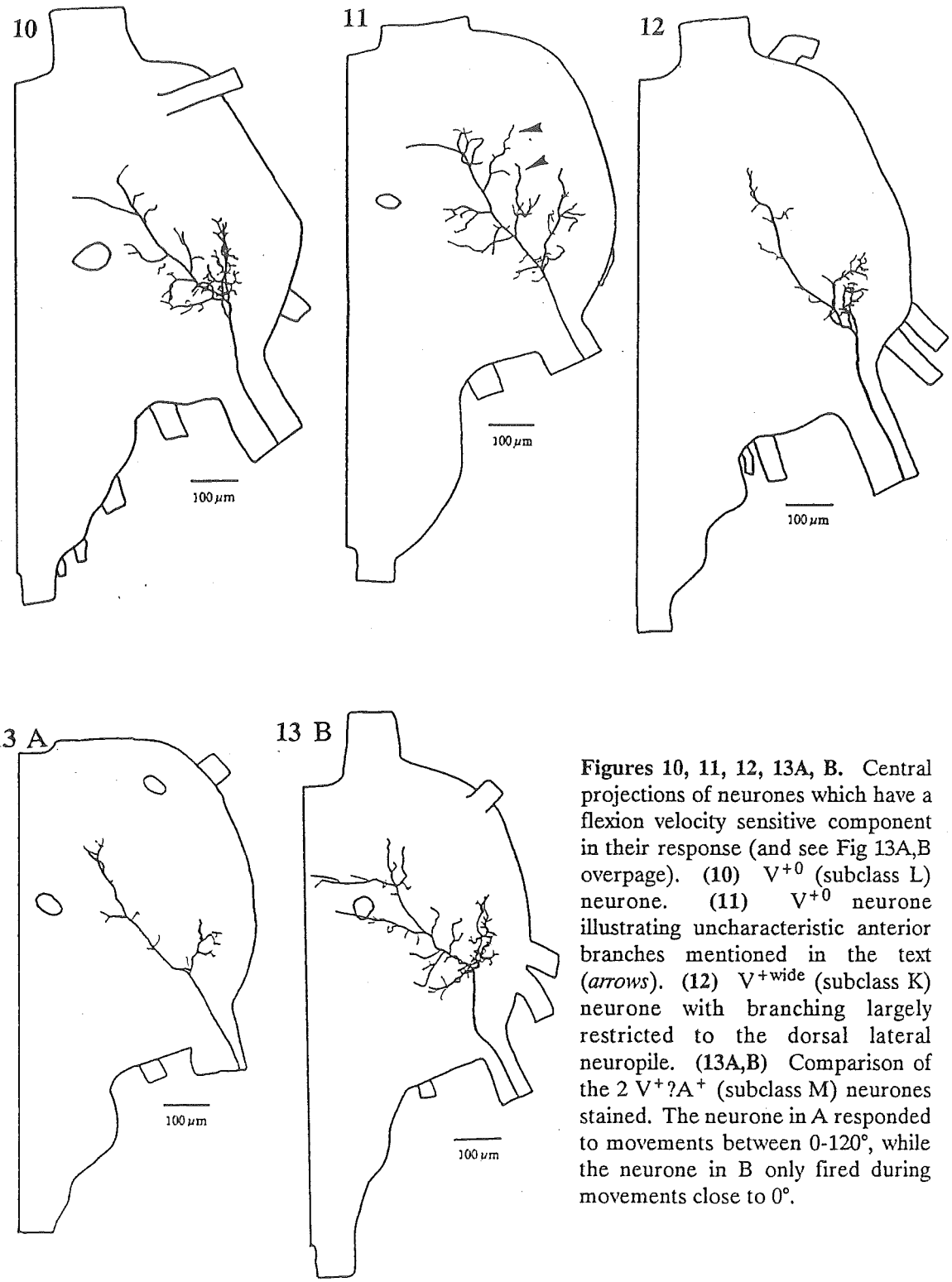
Neurones with a flexion-velocity sensitive (V^+) component in their response were included in classes 3 (P^xV^+), 4 ($P^xV^+?A^+$), and 7 (V^+). Canonical discriminant analysis based on response classes placed them in 3 largely overlapping groups to the left in Fig. 2. The canonical analysis based on subclasses (Fig. 4C,D) provided further separation for some groups.

Pure velocity-sensitive units. Pure V^+ units (class 7, subclasses J-L) are spread out along the vertical axis of Fig. 4C. Units which fire only as the leg reaches full flexion (40-0°: V^{+0}: subclass L) are grouped separately from those which fire over much of the range (V^+: subclass J). Neurones which fire during flexions at all angles (120-0°: V^{+wide}: subclass K) are clustered in a central location, overlapping the other two distributions.

All of the 7 well stained V^{+0} neurones had a short branch in the anterior medial bundle (e.g., Fig. 10). These neurones had a small but dense area of branching in the dorsal lateral region, with few other branches anterior to the main axon. Two neurones were clear exceptions: they had 2 prominent branches extending anterior to the axon (arrows in Fig. 11). One of these neurones had an unusual response: its phasic discharge continued for some time after cessation of the movement stimulus (other V^+ neurones only fired during movements).

While V^- neurones generally had a region of branching anterior and lateral to the base of the branch in the anterior medial bundle (see arrow in Fig. 1), this was poorly developed or absent in V^+ neurones (cf. Fig. 10).

Two V^{+wide} neurones had a morphology similar to that just described for V^{+0} neurones (and were grouped accordingly in Fig. 4A). A third V^{+wide} neurone which responded to movements of less than $20°s^{-1}$ was somewhat different in morphology, and was placed by the analysis among the V^+ neurones. It had a well developed dorsal lateral region of branching but no major branches further medial (Fig. 12). The main axon was well filled with dye, giving no cause to suspect that this pattern was an artifact. This neurone closely resembles some P^xV^+ and $P^x?A$ neurones described later, and had erratic 'tonic' firing similar to that of P^xV^+ or $P^x?A$ units. It was not possible to unambiguously determine if any of this ongoing activity was real tonic firing (because of superimposed bursts of spikes caused by leg tracheal movements), and so the classification as V+ was



Figures 10, 11, 12, 13A, B. Central projections of neurones which have a flexion velocity sensitive component in their response (and see Fig 13A,B overpage). **(10)** V^{+0} (subclass L) neurone. **(11)** V^{+0} neurone illustrating uncharacteristic anterior branches mentioned in the text (*arrows*). **(12)** V^{+wide} (subclass K) neurone with branching largely restricted to the dorsal lateral neuropile. **(13A,B)** Comparison of the 2 $V^{+}?A^{+}$ (subclass M) neurones stained. The neurone in A responded to movements between 0-120°, while the neurone in B only fired during movements close to 0°.

tentative. The morphology suggests that it would be better classified as a phasic-tonic unit.

Three neurones were classified as V^{+} (subclass J in Fig. 4C). Of these, 1 was very faintly stained, 1 had a morphology very similar to that illustrated in Fig. 11 (V^{+0}), and the other had an aberrant morphology, with a branch in each

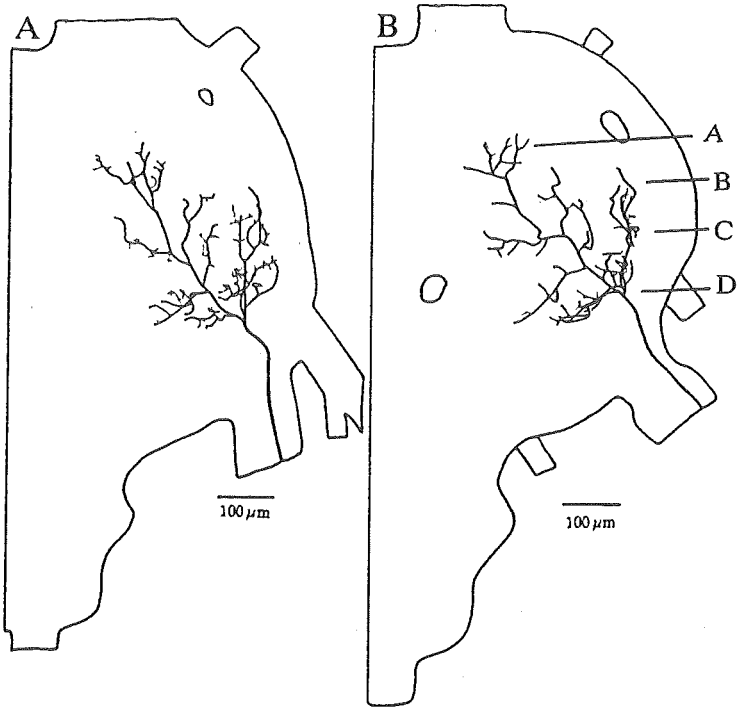


Figure 14A, B. Position-and-flexion-velocity sensitive neurones. The length of the branch in the posterior medial bundle varies between neurones (compare A and B). Sections illustrated in Fig 15 were cut at locations A-D.

medial bundle. Immediately after beginning to stain the neurone recorded from this last preparation, a sudden change in the shape of the injection pulses occurred, suggesting that the electrode had moved out of the recorded cell. I suspect therefore that this stained cell was not the same as that from which the response was recorded. Disregarding this last cell, it can then be stated that V^+ neurones have essentially the same morphology as V^{+0} neurones (In Fig. 4A note the proximity of the 2 lower neurones of subclass J to upper neurones in subclass L.

Flexion-velocity-and-acceleration sensitive neurones. The two $V^+?A^+$ neurones (class 7, subclass M) have very different morphologies from one another (Fig. 13A,B). This is reflected by their wide separation along the canonical axes in Fig. 4C. Both cells were well filled, and clearly characterised so it is difficult to explain the difference. The neurone shown in Fig. 13A occasionally fired a few spikes while the tibia was motionless, but it clearly responded to movements in the range 0-120°. This neurone was far less sensitive to velocity than was the V^{+wide} neurone illustrated in Fig. 12. The other $V^+?A^+$ neurone (Fig. 13B) only responded to movements close to 0°, and was relatively insensitive to velocity.

Position-and-flexion-velocity sensitive neurones. P^xV^+ neurones (class 3 in Fig. 2) comprise subclasses 6, 7, and 8 in Fig. 4A,D).

P^0V^+ neurones (subclass 6) have very short branches in both medial bundles,

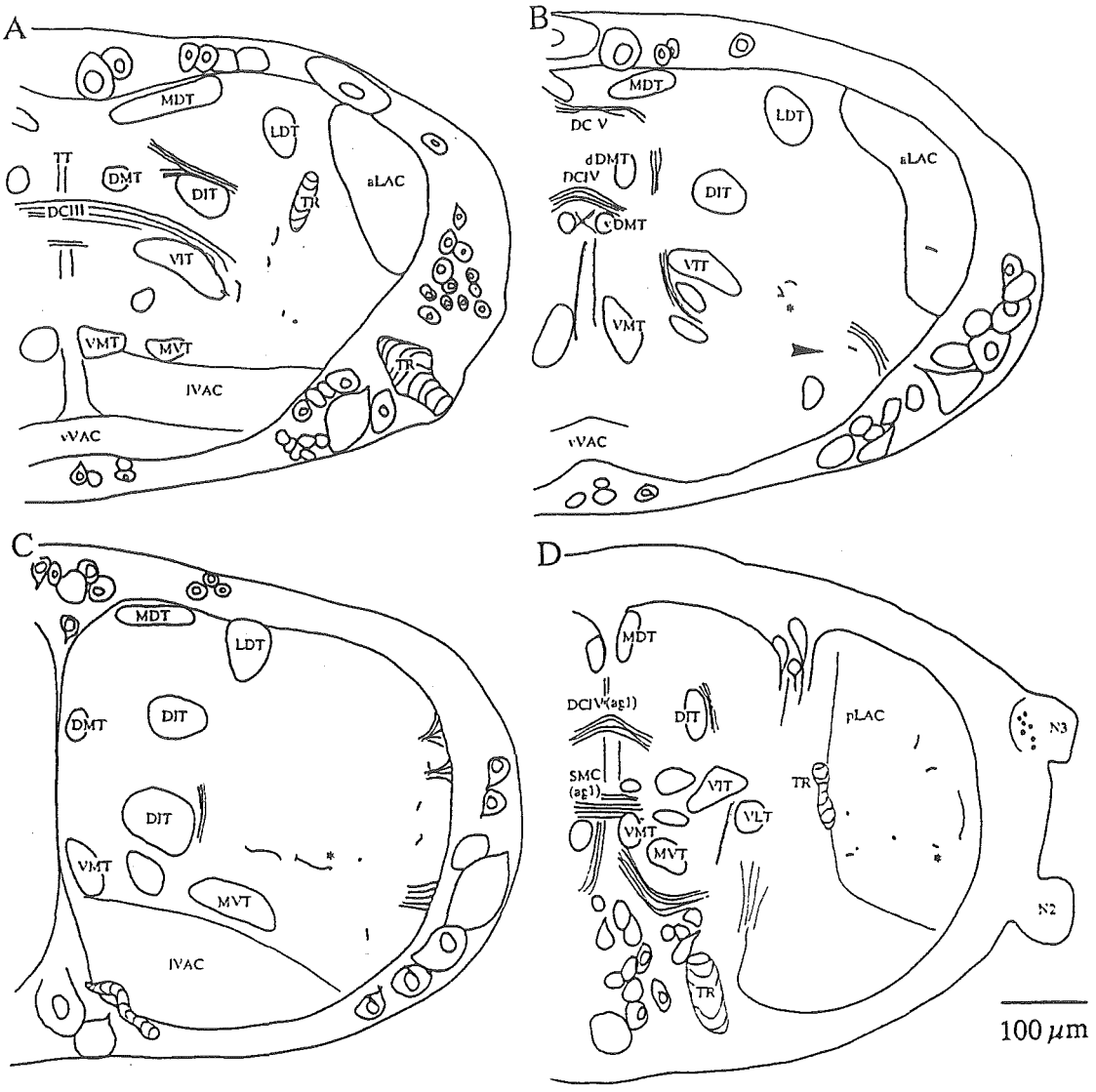


Figure 15A-D. Sections cut through a P^0V^+ (subclass 6) neurone. Each section is discussed in the text. The arrow in B points to a ventral branch of the neurone.

and well developed dorsal lateral ramifications (Fig. 14A). The posterior medial branch is quite variable between neurones, and in some cases appears not to project far enough anterior to join properly into this bundle (Fig. 14B). Anteriorly, in a section cut at location A of Fig. 14B, branches lie lateral to VIT (Fig. 15A). Sections cut at the level of DCIV (Fig. 15B) fail to reveal any medial branches in this case. One lateral branch (arrow) lies ventral to a group of neurites emerging from ventral lateral somata. The anterior-most dorsal lateral branch appears in aLAC. Further back (Fig. 15C) the 'posterior medial' branch can be seen lateral to, and just below the level of DIT. Some dorsal lateral branches are close to

roots of n3. The ventral-most branch continues to run ventral (but lateral) to MVT. Further posterior (Fig. 15D), dorsal lateral and 2 medial branches are apparent in pLAC.

Two P^0V^+ neurones did not have a branch in the anterior medial bundle. In one case this was the result of a poor fill. In the other case, the neurone was well filled, but had only very sparse branching medially, and 2 dorsal lateral branches (Fig. 16). This morphology is therefore very similar to that of the V^{+wide} neurone illustrated in Fig. 12. The P^0V^+ neurone illustrated in Fig. 16 was also very sensitive to movements throughout the leg's arc, responding clearly to velocities as slow as than $20°s^{-1}$ with bursts of spikes. Its response and morphology were quite similar to those of the neurone classified as P^0V^{+wide} (Fig. 17) and those classified as $P^{mid}V^+$ (Fig. 18A,B) (all of which also responded to low velocities throughout the leg's arc of movement). All these neurones should probably be classified together as being relatively insensitive to position, but very sensitive to flexion velocity.

Two neurones which were classified as P^0V^{+0} (subclass 8 in Fig. 4D) were placed slightly lower on the canonical discriminant plot than P^0V^+ (subclass 6) neurones, but there was no obvious subjective difference in their branching morphology from the pattern illustrated in Fig. 14A,B). They are therefore not illustrated separately.

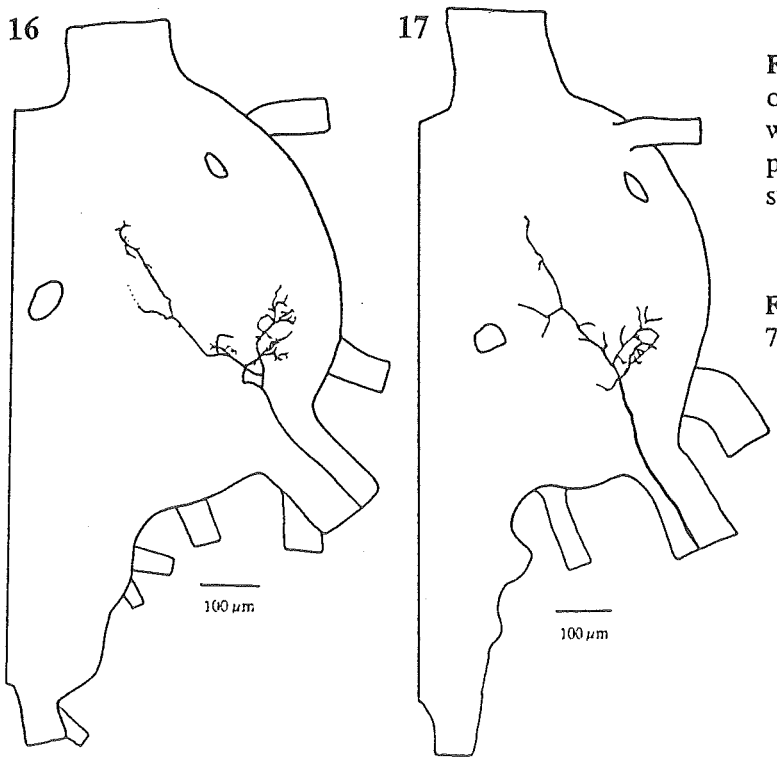


Figure 16. Central projections of a P^0V^+ (subclass 6) neurone with an unusual branching pattern compared to other subclass 6 neurones.

Figure 17. P^0V^+wide (subclass 7) neurone.

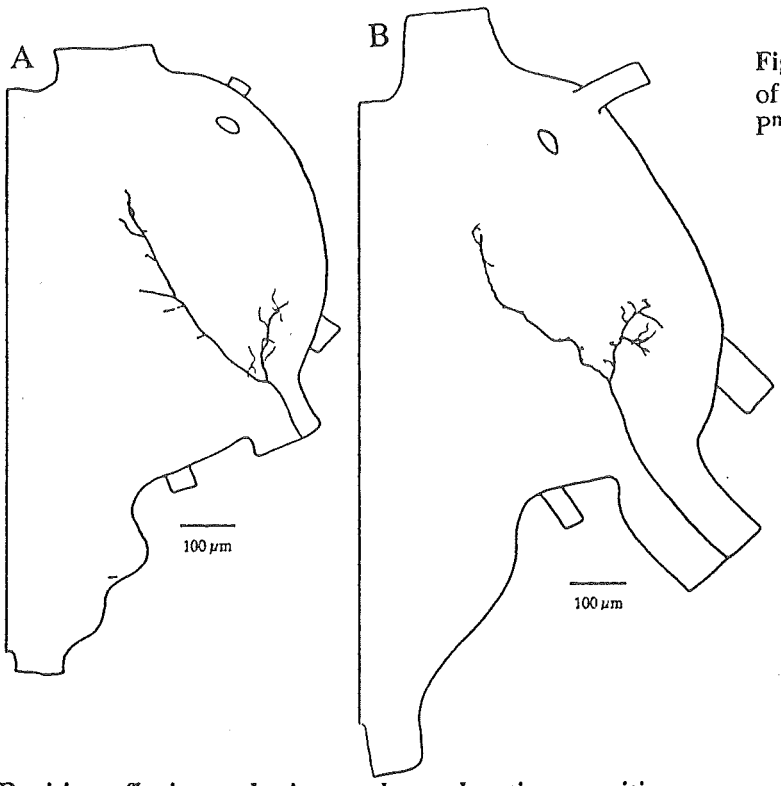


Figure 18A, B. Two examples of the central projections of $P^{mid}V^{+}$ (subclass 9) neurones.

Position,-flexion-velocity,-and-acceleration sensitive neurones. The three neurones in subclasses B ($P^{mid}V^{+}?A^{+}$) and C ($P^{mid}V^{+}?A^{-}$) (Fig. 4A,D) all had a small region of branching in the dorsal lateral neuropile, and no major branches further medial (i.e., the same morphology as that illustrated in Fig. 17 & 18 for $P^{x}V^{+wide}$ units). The wide separation of these groups from each other in Fig. 4A,D appears to be the result of a slightly different path followed by the axon of the $P^{mid}V^{+}?A^{-}$ neurone.

Acceleration sensitive neurones

Pure acceleration units. Neurones with pure acceleration responses (class 1 in Fig. 2) formed a large group overlapping with position-and-acceleration ($P^{x}A^{x}$: class 2) and extension-velocity (V^{-}: class 8) neurones. Pure acceleration units had well developed dorsal lateral branches (the longest of any response class) and a branch in the anterior medial bundle. This general morphology is illustrated in Fig. 19A & B. Sections cut at the locations indicated in Fig. 19A revealed the anterior medial branch in DCIII (Fig. 20A), the dorsal lateral branch anterior to the somata of FETi (Fig. 20B), and ventral branches below the level of VIT (Fig. 20C). A section cut from a similar position in the ganglion of Fig. 19B illustrated these ventral branches reaching as far medially (but ventral to) the centre of VIT (Fig. 20D). Further posterior (Fig. 20E) dorsal branches are evident in pLAC, while some medial branches project towards MVT. A section cut as the main

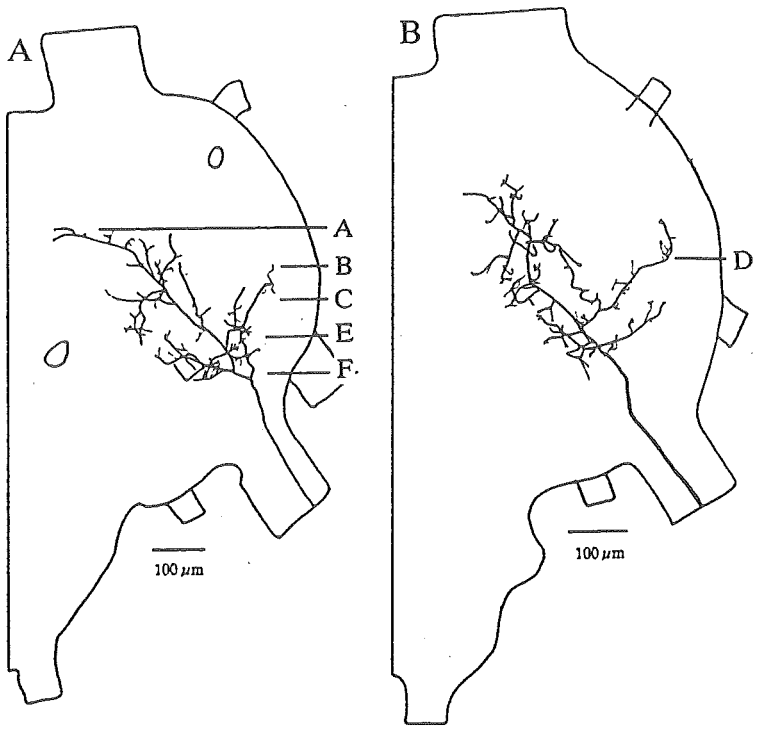


Figure 19A, B. Two examples of the central projections of pure acceleration (class 1, subclass 3) neurones. Sections illustrated in Fig. 20 were taken from locations A-F.

neurite reaches the margin of the neuropile (Fig. 20F) revealed a large medial branch projecting as far dorsal as VLT. A lateral dorsal branch (arrow) reached even further dorsal. All pure acceleration receptors had similar degrees of dorsal lateral branching, and the density of other branches was also fairly consistent. There were however, differences in the lengths of medial branches which related to response classes. These differences must therefore be important in providing the clear separation between subclasses 0, 1, 2, and 3 in Fig. 4E, and are discussed in the following paragraphs.

All 3 neurones which responded only to negative accelerations (A^- (subclass 2) in Fig. 4A,E) had well developed branches in both of the medial bundles (Fig. 21). Four out of the 5 neurones which responded to positive accelerations ($?A^+$ (subclass 1) had a well developed branch only in the anterior bundle (Fig. 22). The other $?A^+$ neurone did not have an anterior medial branch (but was similar in all other respects). Neurones with an ?AS response (subclass 3) exhibited variations between these extremes: one had both medial branches well developed, while others had (to varying degrees) shorter posterior medial branches (n = 8) (these intermediate forms are not illustrated separately because the only apparent difference from the morphology shown in Fig. 21 & 22 is the length of the posterior medial branch).

Position-and-acceleration receptors. Neurones in class 2 (Fig. 2) responded to accelerations and leg position. Subclasses 4 and 5 were based on the angle

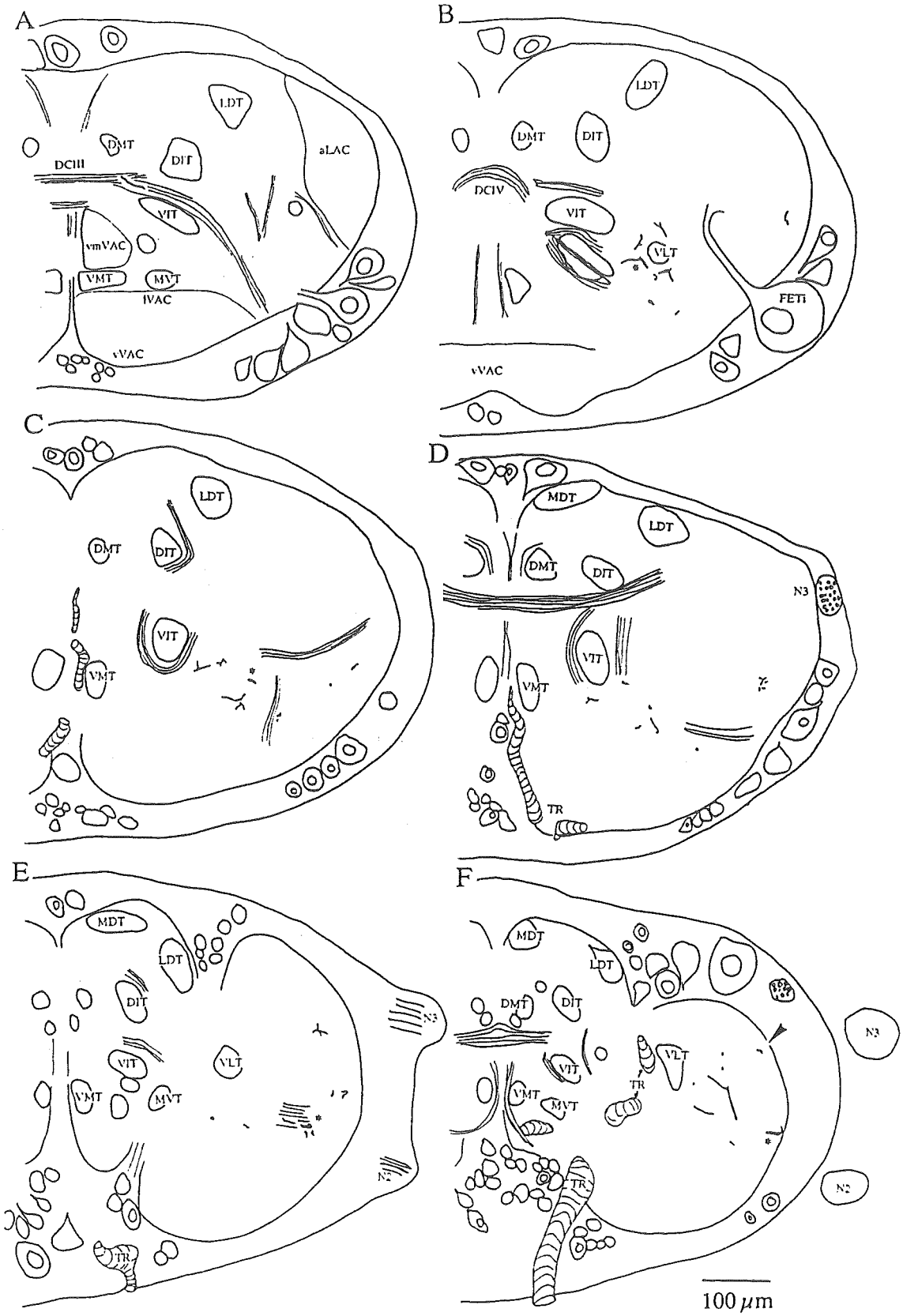


Figure 20A-F. Sections through the pure acceleration (subclass 3) neurones illustrated in Fig 19A,B. Each section is described in the text. Asterisks indicate the main neurite where it can be clearly distinguished from other branches.

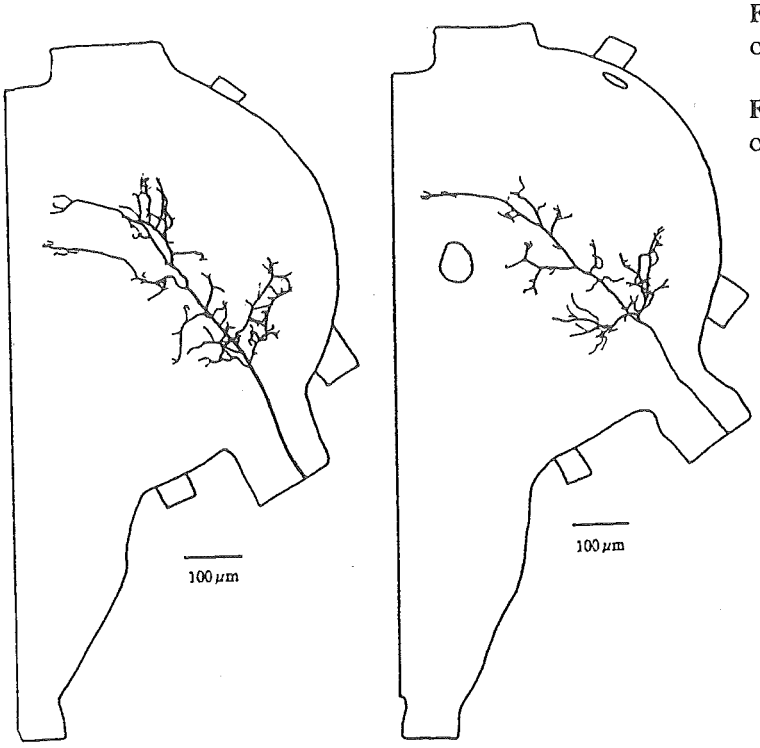


Figure 21. Central projections of an ?A$^-$ (subclass 2) neurone.

Figure 22. Central projections of an ?A$^+$ (subclass 0) neurone.

giving the highest tonic firing (P^0?A = subclass 4; P^{mid}?A = subclass 5). The canonical discriminant analysis based on subclasses was unable to differentiate between these two (note overlap in Fig. 4A,E). There are, however, two distinct morphological types in each subclass, and in retrospect it is possible to attribute these to a different aspect of response: the strength of the relationship between leg angle and firing frequency (i.e., the accuracy with which the firing frequency codes for different static leg positions). Some P^x?A units have a low frequency response (< 5 Hz) which is only weakly dependent on tibial position, while other P^x?A units fire at relatively high frequencies (20-40 Hz), and are strongly influenced by tibial angle.

Neurones (from either subclass) which had low frequency tonic firing (n = 4) had the same branching pattern as pure acceleration receptors (cf. Fig. 22: i.e., well developed dorsal lateral branches, a branch in the anterior medial bundle, and a short (or no) branch in the posterior medial bundle). This pattern is not re-illustrated. None of these units had an ?A$^-$ component, and none therefore had well developed branches in both medial bundles. One other unit had the same response, but a different branching pattern. However, this preparation was ambiguous because 2 cells had been stained with cobalt.

Acceleration sensitive neurones which had high frequencies of tonic firing related to femoral-tibial angle (n = 6) had a completely different morphology to

that just described (compare Fig. 23). They had no main branches leaving the axon anywhere along its length, and no branches in the medial bundles. Sections cut at the locations indicated in Fig. 23 indicated that the main neurite runs medially at about the same level as other FCO axons. In Fig. 24A this neurite can be seen just below the lateral margin of VIT. The most prominent medial branch clearly did not enter DCIV (Fig. 24B). A section further posterior (Fig. 24C) showed that the only significant lateral branch runs slightly ventral to the main neurite, and not dorsal to it as might be expected.

One $P^{mid}?A^{+}$ neurone had a different branching pattern and an intermediate type response (16 Hz tonic maximum), but has been ignored because the quality of the recording declined suddenly just prior to staining (spike size decreased from 30 to less than 5 mV). It is likely that the electrode moved into (and stained) a cell other than the one that was recorded from.

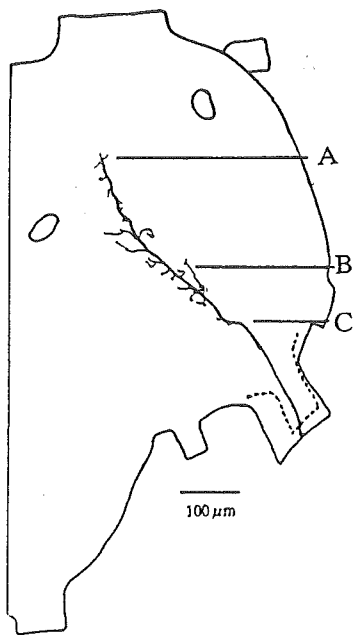


Figure 23. Central projections of a position-and-acceleration (subclass 5) neurone with restricted central branching (and high frequency tonic firing). Sections illustrated in Fig. 24 were cut at locations A-C.

Cluster analysis

The aim of a cluster analysis is to objectively differentiate between observations on the basis of any number of measured variables. In this case, the specific aim was to determine if neurones grouped on the basis of their morphology alone would form clusters related to their responses. If they did, this would allow prediction of a neurones response from its pattern of branching.

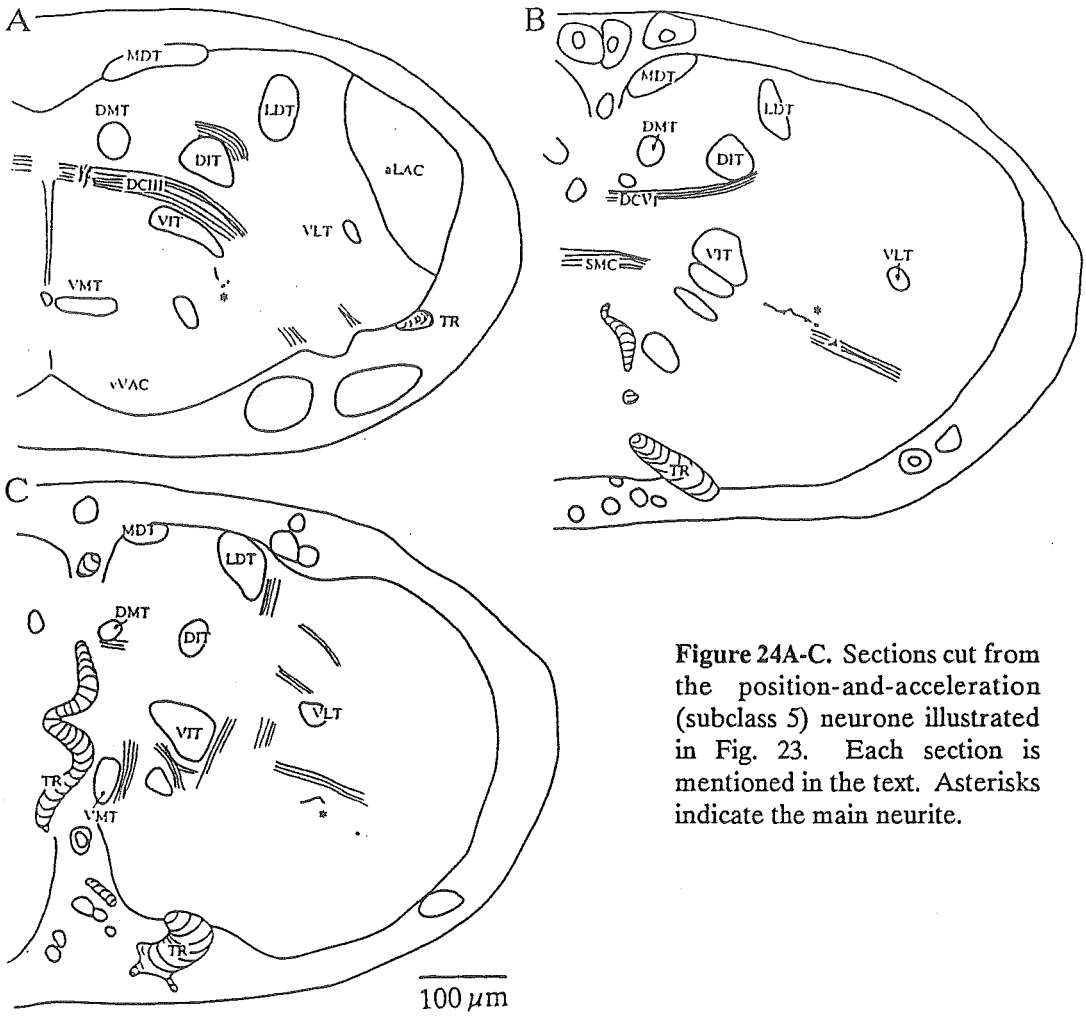


Figure 24A-C. Sections cut from the position-and-acceleration (subclass 5) neurone illustrated in Fig. 23. Each section is mentioned in the text. Asterisks indicate the main neurite.

In Fig. 25 each observation (neurone) is represented by a horizontal line extending leftwards from the right margin. Similar neurones are joined together by vertical lines, their degree of similarity being indicated by the distance along the horizontal axis ("Average distance between clusters"). A distance of 0 would indicate that the neurones were identical. Class and subclass classifications have been added along the right-hand margin to allow a comparison between the clusters and the responses. The vertical broken line at extreme right indicates groups which are separate at an average distance of 0.9. It is valid to split off groups at any arbitrary average distance: generally they are split off at the smallest distance which yields meaningful clusters (all must be split at the same level). In this case, however, moving the cut-off point to the right only increases the number of groups without markedly reducing the variability of response classes within each (i.e., it does not produce better clusters).

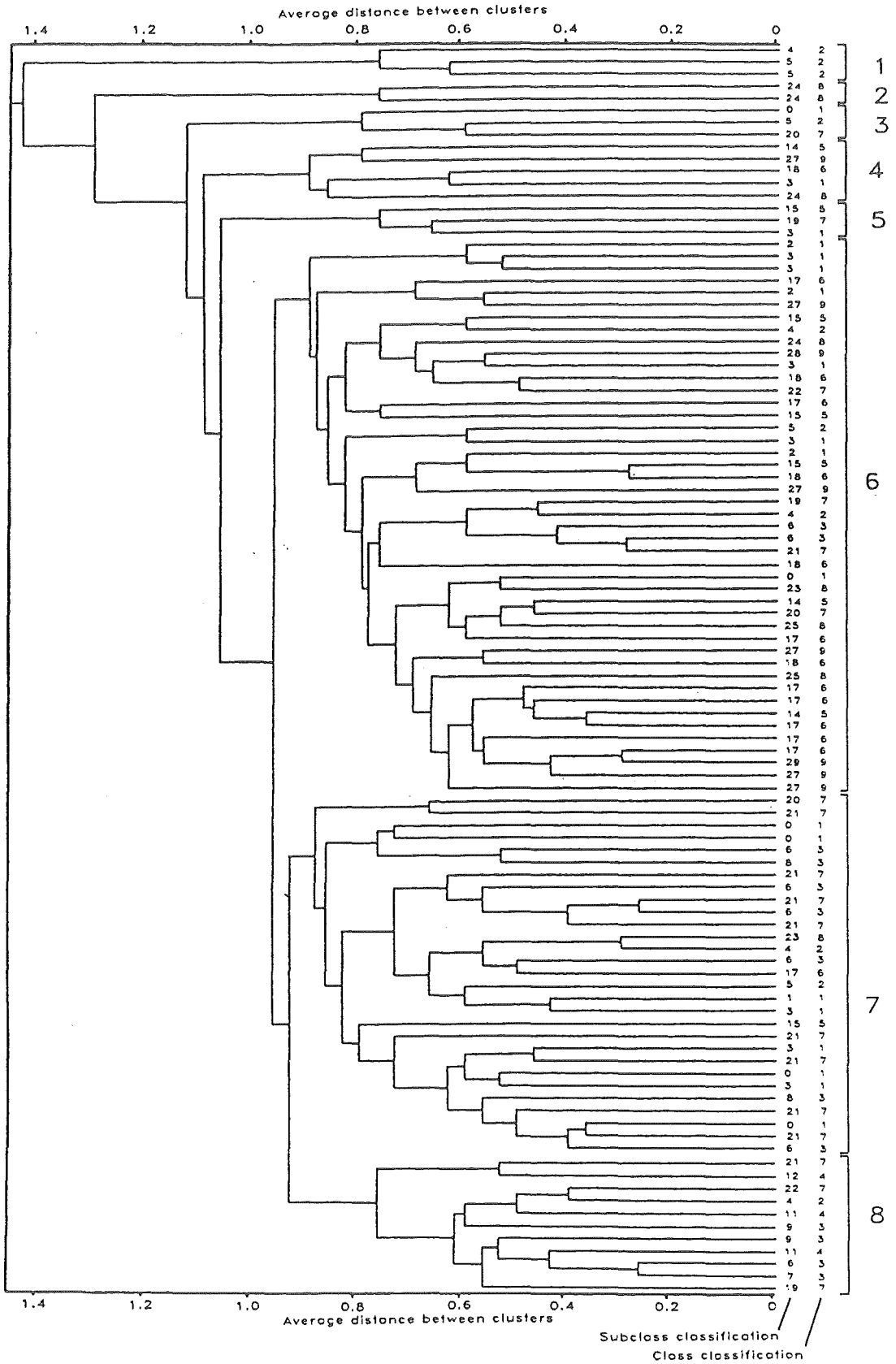


Figure 25. Cluster diagram. Eight main clusters have been split off (numbers 1-8 at far right). Each of these clusters contains morphologically similar neurones (as classified by the cluster analysis. However, it is evident that each cluster contains neurones of many response classes (class and subclass numbers are given inside the broken line to right). Similarity between observations (neurones) or clusters is indicated by the distance along the horizontal where they are joined (average distance between clusters).

Although some clusters contain physiologically significant groups of neurones (e.g., cluster 1 (top)), which contains only class 2 neurones), there is no instance where a cluster contains all the neurones with a particular response, and excludes all other response classes (e.g., although cluster 1 contains just 3 class 2 neurones, 7 other neurones with the same response are scattered in clusters 3, 6, 7, and 8). The cluster analysis was therefore unable to allow prediction of a neurone's response class based on morphological similarities, although the canonical discriminant analysis clearly demonstrated a relationship between response and morphology.

DISCUSSION

In order to understand the functioning and role of sensory systems it is important to understand not only the responses of the individual transducers, but also the functional connections that are made with interneurones and effectors (motor neurones). When investigating a sense organ such as a tactile hair which contains only a single neurone it is relatively easy to characterise and stain the downstream central neurones to which it is connected. Multineuronal sense organ such as chordotonal organs are considerably more difficult to investigate because convergence and divergence of their many afferent signals onto central neurones introduces a great deal of complexity to the system. In addition, it is often not possible to record from individually identified sensory neurones in successive experiments, so each afferent must be morphologically characterised in every experiment.

Because of these complications, even the most thorough studies of the central connections of joint chordotonal organs to date (e.g., Burrows 1987, Burrows et al. 1988, Büschges 1990, Laurent & Burrows 1988) have generally relied on extracellular recordings of the entire organ's response to imposed leg movements, rather than intracellular recordings from single sensory units. This approach allows many more interneurones to be investigated, but precludes interpretation of the specific connections made by individual FCO afferents and identified interneurones. In particular, although the FCO is known to contain neurones which respond to position, velocity, or acceleration, or to combinations of these parameters (Hofmann & Koch 1986, Hofmann et al. 1986, Matheson 1990, Zill 1985a), no studies which have demonstrated central connections of FCO neurones have illustrated the morphology of the afferent neurones. It has not yet been determined if all FCO neurones that provide information about

different aspects of a movement make the same connections with any given interneurone (Burrows et al. (1988). However, it is known that all extension sensitive neurones do not synapse onto 1 particular flexor motor neurone (Burrows 1987).

The work presented here is the first step towards understanding the specific connections of mtFCO sensory neurones. In conjunction with a previous paper (Matheson 1990) it provides the first comprehensive survey of the responses and central projections of these neurones. It is clear from this work that different mtFCO neurones have different branching patterns within the metathoracic ganglion, and that the specific pattern of branches is related to the response of the neurone. Burrows (1987) first suggested that the projections of mtFCO neurones varied, but his 6 published stains were derived from a single whole-nerve cobalt backfill, and were not characterised neurones. In addition, 4 of the patterns that Burrows illustrated have not been recorded in the present survey: they appear to be partial stains because in all cases they have fewer rather than more branches than similar neurones recorded here.

Variation between animals in the central branching of identifiable cricket clavate hairs is considerable (Murphey et al. (1980), although overall there is a clear relationship between peripheral location and branching pattern (see later). Assuming that similar variation occurs in FCO projections (where individual cells cannot be recognised from preparation to preparation on either physiological or morphological grounds) it is evident that in order to elucidate patterns of branching many neurones must be filled before the patterns become obvious. In addition, it is to be expected that there will be exceptional branching patterns caused by developmental 'mistakes' (Altman & Tyrer 1977).

All mtFCO neurones stained in the present study had a main neurite extending as far anterior as DCIII (but not necessarily entering the tract itself). All neurones except the few position-and-acceleration receptors with a high frequency tonic discharge had dorsal branches in the lateral neuropile (aLAC, pLAC). Pflüger et al. (1988) note that mechanoreceptors which have collaterals in these lateral association centres usually lack branches in any of the other well-defined neuropiles (e.g., the ventral association centres (aVAC, pVAC, vVAC). This is apparently the case for femoral chordotonal organs neurones.

Neurones with a branch in the posterior medial bundle (DCIV) always had a branch in the anterior bundle (DCIII), but the converse did not hold true. The length of branches in the medial bundles was related to the range of leg angles in which a given neurone responded: neurones sensitive closer to 120° (extension)

had longer branches. For phasic-tonic (P^xV^-? and $P^xV^-?A^-$) neurones (which all had branches in both bundles) the length of the anterior branch (in DCIII) was more strongly related to response than was the length of the posterior branch. For pure acceleration receptors the length of the posterior branch (in DCIV) was most strongly related to response.

The main aim of the present study was to investigate differences between response classes. An intracellular survey concentrating on a limited number of response classes is now needed to describe in detail the variation within classes. In particular, the 3-dimensional nature of the branching pattern has been largely overlooked in the present study. For the dorsal lateral branches at least (and probably also for some medial branches), variation along the dorsal-ventral axis is likely to be at least as important as anterior-posterior or medial-lateral variation in determining the synaptic connections made by FCO afferents.

Neurones in two response classes had very restricted areas of branching. Position-and-flexion-velocity (P^xV^+) neurones which were relatively insensitive to position, but quite sensitive to the velocity of flexion, had only a few branches in the dorsal lateral region, and no major branches further medial. These neurones must have many fewer synaptic contacts than the majority of FCO afferents. Some phasic-tonic neurones which responded to acceleration, and strongly to position (P^x?A), had similar but more restricted branching: they lacked the dorsal lateral branches. It is somewhat surprising that the P^xV^+ and P^x?A are so morphologically similar, yet have quite different responses.

Neurones which are known to receive connections from FCO afferents include motor neurones, spiking local interneurones (Burrows 1985, 1987), non-spiking local interneurones (Burrows et al. 1988), and intersegmental interneurones (Laurent and Burrows 1988). All of these central neurones have at least some branches in the dorsal lateral neuropiles, and all except the non-spiking interneurones have medially directed branches. It is possible to predict that FCO neurones with restricted dorsal lateral branching are unlikely to make connections with non-spiking interneurones. Tibial flexor muscle motorneurones which are excited by tibial extensions have prominent and dense regions of branching in the posterior medial bundle (i.e., entering or very close to DCIV) (Burrows 1987). FCO afferents which project to this region are all extension sensitive. Some extensor motor neurones also have branches in this region, but they appear not to extend as far medial (compare Fig. 13B,D in Burrows & Pflüger 1988). An intersegmental interneurone excited by tibial flexion has branches restricted mainly to lateral neuropiles, while another which is excited

by both extension and flexion has, in addition, many more medial branches (Laurent & Burrows 1988) which could receive inputs from the extension sensitive FCO afferents which also project to this medial region.

In vertebrates and more recently in invertebrates it has been shown that at least some classes of afferent neurones project into the CNS in orderly arrays. In insects both somatotopic (topographic) and tonotopic mapping have been described. Murphey et al. (1980) showed that clavate hairs on the cercus of a cricket have central branching patterns that are determined by the 'birthday' and peripheral location of each neurone. Older hairs (those appearing at earlier moults) project further into the CNS and have more extensive branches than younger cells. The exact relationship between peripheral position and central branching morphology is not clearly explained. Neurones in the insect visual system (Bate 1978), and auditory receptors (see below) are known to make orderly connections in the CNS. Interneurones postsynaptic to crayfish antennal chordotonal organ neurones have been shown to be arranged in an ordered way in the antennal neuropile (Taylor 1975b). Unfortunately the latter interneurones were simply characterised by extracellular recordings, and no attempt was made to visualise their morphology. The central projections of the upstream antennal chordotonal organ neurones are not known. The most recent example of topographic mapping is that presented in an abstract by Newland (1989): locust mechanosensory hairs on the proximal femur project further anterior in the metathoracic ganglion than do distal hairs on the femur, or hairs on the tarsus. In addition, leg hairs at any given distance from the body project centrally in a way that reflects their position on the circumference of the leg: hairs on the medial surface of the leg project further medial than lateral hairs.

Insect auditory receptors have been studied in great detail. The tuning of cricket crista acoustica neurones determines their projections into the prothoracic ganglion (Oldfield 1983). These neurones possess scolopidia, and are therefore chordotonal neurones by definition. This chordotonal organ, however, does not span a joint in the way that the FCO does, instead it rests on a tracheal airsac apposed to the tibial tympanum. Crista acoustica neurones which are tuned to frequencies lower than 16 kHz project to the anterior of the ganglion, while those tuned to higher frequencies project further posterior within the anterior ring tract (aRT = auditory neuropile). Cells tuned to near the calling-song frequency (16-20 kHz) also have larger central arbours (Oldfield 1983, Römer 1985). Other chordotonal (vibration) receptors from the subgenual and intermediate organs (near the crista acoustica) project to a different region of

the CNS, but also form an orderly array dependent on their frequency response (Römer 1985). In the locust the auditory receptor is the tympanal organ, located in the abdomen (the crista acoustica is part of the tibial tympanal organ in Gryllidae, Gryllacrididae, Tettigoniidea, and Stenopelmatidae). The abdominal tympanal receptors are divided into 4 groups on the basis of their peripheral morphology. Three groups respond to low frequencies, while the other group responds best at high frequencies. All 4 groups project to the aRT, where they arborise in distinct regions along an anterior-posterior axis (Römer 1985, Römer et al. 1988). Neurones which respond best to low frequencies have branches further anterior than do neurones which respond best to high frequencies. In addition, neurones with lower thresholds tend to be further posterior than neurones with higher thresholds at the same frequency (Römer 1985).

The work presented in the present paper is somewhat different to previous reports of orderly projections of afferent fibres in insects. In other cases, the projections are ordered on the basis of either peripheral location (external mechanoreceptors), or on the frequency response (auditory neurones). I have shown that a group of chordotonal receptors comprising a single compact sense organ have central projections which are related to (a) the different modalities of the stimulus (position, velocity, and acceleration) which each monitors, and (b) the angle of the femur-tibia joint which produces maximal tonic or phasic firing. Taylor's (1975b) work on crayfish antennal interneurones suggested that modalities of a stimulus monitored by a chordotonal organ may be represented in discrete locations within the CNS, but the present work is the first to directly address this question for the afferent neurones themselves.

GENERAL DISCUSSION

In order to function effectively in a varied and changing environment, animals must sense aspects of their surroundings, and adjust their behaviours appropriately. It follows then, that animals must also monitor their own behaviours. The wide array of exquisitely sensitive and complex internal and external sensory structures possessed by animals bears testimony to the importance of sensory feedback.

Most animals face comparable biological problems, and many have evolved similar response mechanisms. At a biochemical level, many fundamental physiological processes are identical in animals from all phylogenetic levels. Complex systems too, often function in similar ways in unrelated species. The principles of operation of complex entities such as the nervous system are often hidden by the complexity itself. The goal of studying relatively 'simple' systems (such as the nervous systems of insects) is not simply to understand the system under study, but is also an attempt to elucidate features of operation which can be applied across the spectrum of species. As Bowerman (1977) has pointed out "...generalizations extracted from arthropod preparations will complement that [sic] garnered from the extensively investigated vertebrate preparations...One important consequence of a mechanistic appreciation of the control of arthropod walking is a contribution to the understanding of the neural mechanisms underlying control of human posture and locomotion". This is the frame of reference for my study.

EXTERORECEPTIVE SENSE ORGANS OF INSECTS

In arthropods (for example) single-neurone external receptors (exteroreceptors) include a wide variety of cuticular hairs, some of which respond to touch, vibration, water or wind currents, or even airborne sound (mechanosensors). Other hairs are chemosensory, responding either on contact with a substance, or at a distance (odour receptors). Some basiconic hairs respond to humidity. Campaniform sensilla are dome-shaped structures embedded in the cuticle which respond to stresses, either externally applied, or developed as a result of the animal's own muscular activity. Other neurones located under specialised areas of cuticle respond to temperature. Complex sense organs (internal or external multineuronal structures) include eyes, statocysts (for detection of orientation and movement relative to gravity), haltares (reduced wings and associated sense organs which assist equilibrium during flight), and tympanal (hearing) organs. All these receptors

respond to external stimuli (although some may also respond to the animal's own movements or actions). These receptors allow the animal to sense its surroundings.

PROPRIOCEPTIVE SENSE ORGANS

Sense organs which primarily monitor the animal's own movements or 'state of being' (proprioceptors) include both internal and external structures. External proprioceptors include campaniform sensilla, some hairs near joints, and hair plates (specialised groups of tiny hairs near articulations.

Internal proprioceptors include a range of single-neurone muscle tension receptors and stretch receptors (multipolar neurones), strand receptors with central cell bodies, and multineuronal joint chordotonal organs which detect the movement and position of body segments relative to each other. I have described the morphology, function, and importance of chordotonal organs in the General Introduction.

CENTRAL INTEGRATION OF SENSORY INFORMATION

Information from sense organs (in the form of action potentials moving along a sensory axon towards the central nervous system (CNS)) can be used by an animal in 2 ways. It can be directed immediately to effector neurones (motorneurones innervating muscles), or to interneurones (neurones contained entirely within the CNS, with outputs only on other neurones). In most cases, information probably follows both paths (e.g., Burrows 1987). Direct connections onto motorneurones allow rapid reflexes, while interneuronal pathways mediate complex transformations and comparisons of vast quantities of sensory input. The output from interneuronal pathways is therefore the integrated sum of many inputs (from both sensory neurones and other interneurones), and has the important quality of being highly plastic. Interneuronal pathways can override or modify reflexes, thus allowing animals to respond to external stimuli while maintaining as far as possible their ongoing activity.

PRIOR RESEARCH AND THE CONTRIBUTION OF THIS THESIS

An understanding of "how animals function" in the broadest sense is important not only in its own right as a contribution to the general body of human knowledge,

but also as a basis for applied uses, for example in agricultural or medical fields. My thesis is a contribution towards our understanding of how animals function. It builds on and critically discusses existing knowledge, and goes further to describe and interpret new information about the structure and physiological responses of an important sense organ (the metathoracic femoral chordotonal organ (mtFCO)) in the leg of the locust.

The present work consists of four closely related sections (chapters). The specific findings described in each chapter have been interpreted in detail in their respective Discussions. In this General Discussion I will provide an overview of the entire research project, and make some additional comments concerning directions that future research should take. For the sake of clarity, published works which have been cited earlier in the thesis are not cited specifically again here. Other works, or novel interpretations introduced for the first time in this section are given full citations.

Chordotonal organs have been the focus of much interest because they are complex sense organs which are readily accessible in robust and behaviourally interesting animals, the arthropods. In addition, the subgroup of chordotonal organs which monitor joint movements must have similar functions to analogous joint receptors in vertebrates. The control of insect walking has received much attention from workers involved in the development of multi-legged robots. Machines which use legs to move must overcome the same problems as insects walking over complex terrain. An understanding of arthropod coordination provides important insights into possible mechanical solutions. Study of the sense organs influencing the control of insect stepping is an important part of this research.

Despite the volume of published work concerning chordotonal organs, there were clearly a number of questions which required investigation. The general morphology and development of many chordotonal organs (including the mtFCO) have been described (Slifer 1935, Mill 1976), but there was still some doubt about the number of neurones in the locust mtFCO. It had been proposed that this organ was reduced in size and generally less important than the homologous organs in the anterior legs. My Ph.D. supervisor, Dr Larry Field had for some time suspected that this was not the case, and we decided that this question would be the starting point of my investigation.

CHAPTER ONE: mtFCO MORPHOLOGY.

The first chapter of my thesis is a redescription of the anatomy of the locust mtFCO, published as a jointly authored paper (with Larry Field). Previous descriptions of the anatomy were shown to be inaccurate because they did not include a substantial group of small neurones distal to the known neurone somata. Our findings suggest that this group represents part of the FCO (the proximal scoloparium) which had previously been thought to be missing in the hind leg chordotonal organ (compared to those of the fore- and midlegs). The implication of this is that the position and movement of the hind leg are monitored as accurately as they are in the anterior legs. Prior to our study it had been believed that because the hind leg was not used extensively during walking it was monitored and controlled less accurately.

CHAPTER TWO: RESPONSES AND LOCATIONS OF FCO NEURONES.

Having re-described the anatomy of the mtFCO, I set out to investigate how individual receptors responded to imposed movements of the apodeme. I had 2 goals: the first was to extend the anatomical findings by determining the responses of the small distal neurones; the second was to map the locations of physiologically characterised neurone somata in the FCO.

In insect crista acoustica (auditory organs in the forelegs which are composed of chordotonal sensilla), the neurones are ordered in a size-dependent array (e.g., Ball & Field 1981). A neurone's position in the array also reflects its tuning to various sound frequencies: proximal cells respond better to low frequency sounds, while distal cells have lower thresholds to high frequencies (Oldfield 1982). The sensitivity of different crista acoustica receptors was known to be determined at least partially by mechanical constraints of the supporting tissues, but the frequency response appeared to be characteristic to individual neurones (Oldfield 1985). Similarly, it was known that receptors in crustacean chordotonal organs were arranged according to their response (sensitivity to either stretch or relaxation) (Hartmann & Boettiger 1967).

Although the physiology of insect femoral chordotonal organ neurones had been studied in greater detail than that of their crustacean counterparts (they were known to respond to static position, velocity, or acceleration of movements, or combinations of these parameters), the arrangement of identified receptors

remained to be elucidated. It was therefore of great interest to examine the positions of neurones with characterised response types. Zill (1985) had begun this task, but did not succeed in characterising cells adequately, and he did not publish a map of cell locations: instead he presented only textual descriptions. The responses of FCO neurones in the stick insect had been thoroughly studied, but there was no attempt to determine the locations of the cell bodies.

I was able to confirm that locust mtFCO neurones had generally similar response types to neurones in the stick insect FCO. The overall response of the organ is complex. It responds to a wide range of velocities, to static position, and to accelerations. The large number of neurones within the organ allow range fractionation, and specialisation of receptor modalities (i.e., all neurones do not respond to all parameters of a movement. The locust has accurate control over the position of its legs, and can be trained to maintain a set angle (Forman & Zill 1984). The sensitivity of the mtFCO is clearly an important part of the control system.

In doing so, I presented the first detailed maps of characterised FCO neurone positions. Zill's (1985a) generalisations concerning the mapping of neurone somata within the FCO were shown to be largely inaccurate. In particular, both flexion and extension (stretch and relaxation) sensitive neurones were shown to be distributed throughout the organ rather than discretely grouped as he had suggested. Some neurones were shown to respond to relaxation, a possibility that Zill had already pointed out. An understanding of the mechanical organisation of the FCO is an important contribution to the ongoing debate about the mechanisms of mechanosensory transduction in scolopidia. A variety of models have been proposed to explain differential sensitivity (e.g., stretch versus relaxation sensitivity). Zill claimed that extension-sensitive neurones had scolopidia lying at an angle to the FCO main ligament, while flexion sensitive neurones had scolopidia aligned parallel to its axis, thus providing a basis for differential activation. My work has shown this not to be the case: both types had scolopidia lying in a variety of orientations. Differential activation must be attributed to some other mechanism. Already Field (in press) has used this information in developing a mechanical model of the basis of range fractionation in the locust mtFCO.

In this phase of my study I had hoped to describe the responses of the newly described distal neurones. This proved to be difficult because intracellular recordings could not be obtained from their tiny axons. I attempted to record

from their somata, but still could not obtain adequate responses. In order to isolate the FCO from tracheal movements so that stable intracellular penetrations could be obtained it was necessary to manoeuvre a platform beneath it. This introduced 2 problems: (1) the FCO was distorted slightly; and (2) meaningful stimulation of neurones by stretch or relaxation of the ligament was hindered by contact with the platform. As noted by Zill, recordings from FCO somata preclude any significant movement of the apodeme because the neurone will be pulled off the recording electrode. I made several attempts to isolate the small branch of the cuticular nerve which carries the distal neurone's axons. It proved to be too short and too closely integrated into the outer lamella of the FCO to allow even a tiny hook electrode to be placed under it.

It is possible to speculate that if these distal neurones have retained the same function as neurones in the proximal scoloparium of the forelegs, then they should respond to vibrations, and have relatively little input to reflex circuits (Field & Pflüger 1989). Field (in press) has recently suggested that the attachment cells of these distal neurones comprise the side (s) bundle of fibres in the FCO ligament. This part of the ligament is mechanically specialised to provide differential tension on each strand, and can thus act as a basis for range fractionation of neurone's responses. This observation is at odds with the prediction that these distal neurones respond to vibrations: if this was the case they would have no need for range fractionation, and in order to respond at all leg angles their attachment cells should remain taut throughout the leg's arc of movement. In the stick insect FCO (and in most other joint chordotonal organs), tonic neurones are smaller than phasic units. Determination of the responses of distal neurones seems therefore to await an experimental solution.

CHAPTER THREE: RANGE FRACTIONATION AND HYSTERESIS.

The demonstration that the locust mtFCO contained a wide variety of response types led directly to the last phase of my experimental work: a determination of the central projections of characterised neurones (Chapter Four). Because these experiments, like those in the last section, involved making recordings from FCO neurone's axons, it was possible to pool all the responses into a single large dataset for an analysis of range fractionation and hysteresis.

Both of these phenomena have been described in many sensory systems, including chordotonal organs of crustacea, and insect auditory organs, but a

thorough study of insect joint chordotonal organs was lacking. I made use of my substantial database to illustrate the responses of many tonic and phasic neurones; to summarise the overlap between response ranges; and to make some inferences about mechanisms of hysteresis. The information in this section detailed in greater depth the existence of mid-position sensitive neurones first reported from the locust mtFCO in my previous chapter. This confirmed and considerably extended the comparable results presented by Hofmann et al. (1985) for similar units in the stick insect FCO. I suggested that extracellular recordings of the massed discharge of FCOs (used in many previous studies) have hidden the responses of smaller and less common neurones, especially mid-position sensitive units. The summed firing frequencies recorded using extracellular techniques are much lower than would be predicted from the known firing rates of individual receptors. Extracellularly recorded responses may therefore bear little resemblance to the actual input to the CNS.

I discussed problems associated with trying to formulate a viscoelastic model of the FCO. Such a model could help explain the observed non-linearities in neurone's phasic and tonic responses. I suggested that direct measurement of the forces transmitted to the neurones would be more direct than attempting to measure the various viscoelastic variables. Interactions between position and velocity responses, and the mechanical constraints underlying range fractionation are clearly important phenomena which require additional investigation. My study has begun this task by describing in detail the many response types present in the mtFCO.

CHAPTER FOUR: CENTRAL PROJECTIONS.

Previous workers have shown in several cases that sensory afferents project into the CNS in orderly arrays. For example, individual scolopidial sensilla from insect auditory organs are known to project into the auditory neuropile of the CNS in an orderly array related to their frequency response. The central projections of tactile hairs on the legs of locusts, and on the cerci of cockroaches are mapped topographically within central ganglia so that their central morphology reflects the position of the hair on the appendage. One step deeper in the CNS, interneurones which respond to different aspects of a movement monitored by chordotonal sensilla in the antenna of a crayfish are arranged in groups according to their response. The 2 scoloparia of the FCOs in locust forelegs respond differently to stimuli, and are known to have distinct regions of branching.

Variation within the 2 groups has not been investigated. It has been suggested however, that different receptors within the mtFCO have different branching patterns within the metathoracic ganglion. No attempt had previously been made to investigate this important proposal. Because the responses of many FCO neurones were now characterised and known to fall into response classes (Chapters 2 and 3) I was in a strong position to investigate and interpret their individual central projections. This was the fourth and final goal of my research. To my knowledge, it was the first study of its type in a joint chordotonal organ.

I showed that neurones belonging to different response classes had distinguishable patterns of branching, and that within at least some classes neurones were arranged according to the range of leg angles which elicited the strongest responses. This is the first demonstration of an orderly projection of neurones from any chordotonal organ related to a stimulus parameter other than frequency of vibration. These observations strongly imply that different mtFCO afferent neurones make connections with different motor- and interneurones, and therefore have various roles in the generation of reflexes and complex behaviours.

This section of my work also described a novel use of statistical analyses as a basis for objectively categorising and describing neuronal branching patterns. The usefulness of the various methods is discussed. A cluster analysis proved to be ineffective at predicting a neurone's response from a known pattern of branching in this case. Canonical discriminant analyses demonstrated that the branching patterns of related FCO neurones were similar. This technique provided a useful basis for describing and grouping the neurones on morphological grounds, and facilitated a discussion of the relationships between response and morphology. Both techniques could be profitably applied to many other studies of neural anatomy.

FUTURE RESEARCH: WHAT QUESTIONS NEED TO BE ANSWERED ?

My research has provided solutions to a number of questions, and has paved the way for several new ones: such is the progress of science.

It seems that each new study of the mtFCO reveals some new complexity: the present work is no exception. The revelation that both stretch and relaxation sensitive neurones are distributed throughout the organ, and respond to movements of the apodeme, suggests that the flexor strand has an indirect (if any) role in stimulating mtFCO neurones. This ligament is known to contain a separate

stretch receptor neurone. Is it possible that the strand's main role is to activate this neurone, and that effects on the FCO are largely incidental ? On morphological grounds (Field in press) this would seem to be the case.

The large posteriorly directed attachment to the flexor muscle which was mentioned in the General Introduction has never been examined in detail, but it certainly exists - contrary to Zill's observations. Burrows (1987) and Bräunig (1985) both illustrated this attachment. The latter author termed it *vlig* (ventral ligament). It is considerably larger in crossection than the flexor strand, and seems to distort the FCO as the leg reaches full flexion. It is relaxed for leg angles between 140 and 50°, but progressively tightens between 50 and 0° (Pers. obs.). When the leg is fully extended, the point of attachment to the flexor apodeme is approximately opposite the FCO. As the leg flexes, the attachment point is pulled proximally. No-one seems to have investigated the possibility that vlig could cause firing of mtFCO neurones, and yet it seems likely (on morphological grounds) that it could exert considerably more force than the flexor strand. The role of this structure needs urgent attention.

The viscoelastic properties of the FCO ligaments must affect the force transmitted to the scolopidia, and thus influence the responses of neurones to tibial movements. In light of Field's recent description of a mechanical basis for range fractionation in the mtFCO, and my observation of non-linearities in responses to linear stimuli, a study of the composition and mechanics of individual attachment strands is in order. The first task should be to describe the overall transmission of energy along the strand (i.e., how is the initial stimulus modified by the properties of the strand). It should be possible to directly measure tension close to the FCO.

The responses of the newly described distal neurones have not been recorded. Such recordings would be a valuable test of our hypothesis that these neurones represent the 'missing' proximal scoloparium. Although technically difficult, intracellular recordings appear to be the best approach. Recording from somata would at least determine whether these neurones had tonic discharges at given angles. It may also be possible to test their response to small amplitude, high frequency vibrations. Recordings from their axons are clearly required for a complete characterisation. Evidence to date suggests that their spikes will be very small, thus precluding extracellular techniques. Because of my desire to thoroughly characterise FCO neurones, intracellular recordings which had small unstable resting potentials and spikes were usually passed-over in favour of

stable recordings which could be maintained for long periods of time. A study with the sole aim of characterising distal neurones should concentrate on these 'poor' recordings, even if the degree of physiological characterisation is somewhat limited.

My study of FCO central projections clears the way for studies of the connections made within the CNS by individual FCO neurones. Although laborious, such studies would have great rewards. Now that at least some of the relationships between FCO neurone's responses and central projection patterns have been described, these studies of connectivity could concentrate on recording from single downstream neurones while sampling as many FCO inputs as possible. The motorneurone or interneurone could be stained at the end of the experiment, and its pattern of branching compared to the pattern of branching expected for each of the sampled FCO neurones (from my work).

SUMMARY

1. The locust metathoracic femoral chordotonal organ contains on average 92 neurones, approximately twice as many as previously described.

2. These small distal neurones appear to represent the 'missing' proximal scoloparium; with the consequent implication that the mtFCO is likely to be as important as the homologous organs in the forelegs.

3. Both stretch- and relaxation-sensitive neurones are distributed throughout the FCO. Both types of neurone are activated by movement of the apodeme while the flexor strand is held motionless.

4. Neurones with similar response types have somata in comparable locations within the FCO, and have similar central projections.

5. Range fractionation of mtFCO neurone's responses is confirmed: at least some units could act a labelled lines to signal when the leg approaches either full extension or flexion.

6. Units which have clear mid-position sensitivity are described.

7. Neurones within at least some response classes have central projections which are ordered with respect to a novel parameter: the leg angle which causes the greatest tonic or phasic firing.

ACKNOWLEDGEMENTS

Many different people have contributed to this thesis in a variety of ways. I wish to thank them all for their help, and for making the last few years so enjoyable.

My supervisor, Dr Larry Field, has always been very enthusiastic about my research. Our stimulating discussions about all aspects of the work (and about neurophysiology in general) have been most enjoyable. I hope for my part that I have managed to lift the gloom of exam and essay marking from over his head on occasions ! I especially wish to thank Larry for faithfully bounding out of his office whenever I turned up the audio monitor to blare out a particularly 'juicy' recording. Pavlov would have been most impressed. My associate supervisor, Dr Harry Taylor, gave me valuable help when I was attempting to set up a computerised data acquisition system, and gave me the run of his computer. Many thanks Harry. I also appreciate the interest that other members of the academic staff have shown in my work.

Jan McKenzie guided me through the electron- and light-microscope parts of the work, and was always around to help with microscopes, cameras, and other stuff. Jan, you do far too much for everyone, but many thanks anyway. Franz Ditz designed and built the ramp generator which was vital to my research, and fixed various other equipment in my times of need. Tracey and Lyn always had something to say, and Lyn especially, always reacted so well to provocation of the humerous sort. Lyn, I hope that my barbaric treatment of the locusts hasn't scarred you for life: it would be a great shame at such a tender age. Other members of the technical staff gave me help at various stages: thanks to Graeme Bull, Tas Carryer, Sandy Gall, Dave Greenwood, and Terry Williams.

Joachim Pflüger (Konstanz) provided useful comments on a draft of the first paper (Chapter One), while anonymous referees suggested several valuable modifications to both papers (Chapters One and Two).

Russell Death has been around here as long as I have, and has always been amenable to discussing physiology. Not bad for a community ecologist. His considerable statistical expertise was given willingly when I was trying to come to terms with the analyses in this thesis (those grumbles were just for show weren't they Russ ?). The rest of the crowd (both from University, and from outside: sorry, I'm not going to list you all) has always been a lot of fun, and have helped me to keep a perspective on things (albeit a somewhat twisted perspective). I'd like to thank all those people who were the butt of my various attempts to get a

laugh, especially Allen Rodrigo who worked on our non-existent Vax for at least 10 min before wondering why everyone was rolling on the floor in laughter; Liz Halsey for spilling the beans about her sex life; and Andrew LeBeau for being such a great target all the time. Rachel and Rebecca never did pose with the beach-balls, but were great company anyway.

My various room-mates have kept things alive with their varied personalities: Curt Lively ('Coitus' to Jan) continually surprised me by speaking when I thought that he was sound asleep; Siân Forlong-Ford introduced a touch of class and glamour to the office; and Dr Neil Hamilton forced more than a few litres of alcohol past my tonsils. Thanks to you all.

Over the last 3 years I have been involved with the production of the journal *New Zealand Natural Sciences*. This has been a great learning experience, and has been an important part of my Ph.D. I would especially like to thank Dr Mike Winterbourn for his contribution as Chairman of the Editorial Board. He always had good ideas when they were needed. It is a shame that some other members of the staff don't have the same enthusiasm for the Journal that he clearly does.

My parents have always been supportive of my efforts, even when I didn't write home for months at a time. Their attitudes towards education have been important in shaping the way that I approach my work.

My final vote of thanks must go to my wife Helen, who slaved away earning money while I slaved away earning very little. Helen put up with my computer, and with my habit of always arriving home 30 minutes later than I said I would. Over the last few months especially, the household would have fallen apart from neglect if it weren't for her efforts. I hope the trip to Cambridge was worth the wait.

APPENDIX I

Program to control analogue ramp generator

The analogue ramp generator used to provide movement stimuli to the FCO in my study was designed by Franz Ditz (Technician, Zoology Department, University of Canterbury). It produces linearly increasing, decreasing, or standing voltages. The output from the ramp generator drives a power amplifier which in turn drives the Ling vibrator. There is no feedback within the system. Initial calibration of displacement was carried out under a binocular microscope fitted with an eyepiece graticule (as described in Field & Burrows 1982). Front panel controls allow alteration of the sign of the ramp (momentary-contact bipolar switch), the magnitude of the output voltage (variable potentiometer), and the slope of the ramp (range selector switch and variable potentiometer). The voltage can also be set temporarily to 0 V (momentary-contact switch), or reset to 0 V (momentary-contact switch). A linear ramp is produced by setting the appropriate slope rate, and holding the momentary-contact bipolar switch closed towards the side producing the desired sign of slope. When the switch is released, the voltage remains set at the final level until a further stimulus is given, or until it is reset to 0 V. In practice, the magnitude of the output voltage was never altered, as this was set to a voltage equivalent to a complete 0-120° leg movement for all experiments.

In addition to the manual controls, the ramp generator has relays to allow external voltage pulses from the serial output port of an IBM compatible computer to control the onset, sign and duration of ramp stimuli. In practice, manual stimuli were used only occasionally to test 'aberrant' neurones, or to check the equipment: stimuli used for data collection were computer controlled, thus allowing greater accuracy and reliability. I designed and wrote the following program in Turbo BASIC to run the equipment. Details of its use and design can be found near the end of the listing (in the 'Help' section). Briefly, the program keeps track of the present voltage (leg angle), and allows the user to set up staircase stimuli in which the initial sign, the slope, and the duration of the steps and intermediate 'holds' are variable. The program calculates the appropriate front panel settings, and prompts the user to make the necessary adjustments. A key press initiates the stimulus, which consists of an accurately timed series of pulses to control the beginning and end of each step in the staircase. At the end, the stimulus can be modified or repeated. All user input to the program is in response to clear on-screen prompts. There is provision to save and retrieve

stimulus protocol files on disk. A help facility is included. The program is used in its compiled form (i.e., as an executable file) so that a copy of Turbo BASIC is not required. The executable file is 59968 bytes long.

LISTING

```
'PROGRAM TO CONTROL AN ANALOGUE RAMP GENERATOR VIA THE RS232 SERIAL PORT
'THE OUTPUT LINES DTR AND RTS ARE USED TO SUPPLY PULSES WHICH ARE
'CONVERTED INTO TTL PULSES TO AFFECT RAMP POLARITY, RAMP DURATION
'AND HOLD DURATION. THE RAMP VELOCITY IS SET BY A POTENTIOMETER
'ON THE FRONT PANEL OF THE RAMP GENERATOR ITSELF.

'WRITTEN BY TOM MATHESON, 1988.

COM1 = &H3FC 'THIS IS THE MEMORY LOCATION TO USE WHEN SET FOR COM1
COM2 = &H2FC 'THIS IS THE MEMORY LOCATION TO USE WHEN SET FOR COM2

ON ERROR GOTO ERRORTRAP              ' CATCHES MAINLY DISK ERRORS

PORT = COM1
OUT PORT,0
CLS

'*****************************************************************************
'DEFINE CONSTANTS
'*****************************************************************************

MAXANGLE = 120                       ' DEGREES
MINANGLE = 0                         ' DEGREES
MAXVELOCITY = 1000                   ' DEGREES/SECONDS
MAXARC = 120                         ' DEGREES
MAXTOTALTIME = 60                    ' SECONDS
SETTLINGTIME = 10                    ' SECONDS DELAY FOLLOWING SETTING UP THE START ANGLE

'*****************************************************************************
'DEFINE VARIABLES
'*****************************************************************************

CHECKEDFLAG$ = "NO"                  ' OR "YES" - ALLOWS OR DISALLOWS VALUES TO BE SAVED
POLARITY$ = "POS"                    ' OR "NEG"
STARTPOLARITY$ = "POS"               ' OR "NEG"
UNIPOLAR$ = "N"                      ' OR "Y"
STEPS = 1                            ' NUMBER OF STEPS IN 1 HALF OF THE MOUNTAIN
VELOCITY = 600                       ' DEGREES PER SECOND
HIGHANGLE = 120                      ' UPPER LIMIT FOR THIS RAMP
LOWANGLE = 0                         ' LOWER LIMIT FOR THIS RAMP
STARTANGLE = 0                       ' START ANGLE, GIVEN HIGH AND LOW LIMITS AND POLARITY
ENDANGLE = 120                       ' END ANGLE, GIVEN HIGH AND LOW LIMITS AND POLARITY
CURRENTANGLE = 60                    ' KEEPS A TRACK OF THE CURRENT ANGLE
ARC = 120                            ' DEGREES OF MOVEMENT FOR THIS STEP
RAMPDURATION = 0.2                   ' SECONDS
HOLDDURATION = 0                     ' SECONDS

'*****************************************************************************
'FIRST MENU
'*****************************************************************************

FIRSTMENU:

CLS
LOCATE 1,16
PRINT"+=========================================+"
LOCATE 2,16
PRINT"| CONTROL PROGRAM FOR RAMP GENERATOR |"
LOCATE 3,16
```

```
PRINT"|       BY TOM MATHESON 1988       |"
LOCATE 4,16
PRINT"+=========================================+"

LOCATE 7,20
PRINT "1: Generate a ramp."
LOCATE 9,20
PRINT "2: Save current parameters to disk."
LOCATE 11,20
PRINT "3: Read parameters from disk."
LOCATE 13,20
PRINT "4: Manual reset to 0 volts."
LOCATE 15,20
PRINT "5: Help."
LOCATE 17,20
PRINT "6: Quit program."
TRYAGAIN:

LOCATE 19,7
PRINT"Type a number or CAPITAL letter corresponding to your choice. "
CHOICE$ = INPUT$(1)
CHOICE = VAL(CHOICE$)

IF CHOICE > 6 OR CHOICE < 0 THEN
        GOTO TRYAGAIN
ELSEIF CHOICE = 0 THEN

        IF CHOICE$ = "G" THEN
        CHOICE = 1
    ELSEIF CHOICE$ = "S" THEN
        CHOICE = 2
    ELSEIF CHOICE$ = "R" THEN
        CHOICE = 3
    ELSEIF CHOICE$ = "M" THEN
        CHOICE = 4
    ELSEIF CHOICE$ = "H" THEN
        CHOICE = 5
    ELSEIF CHOICE$ = "Q" THEN
        CHOICE = 6
    ELSE
        GOTO TRYAGAIN
    END IF

END IF

CLS
ON CHOICE GOTO GENERATE,SAVEPARAM,READPARAM,MANUALRESET,HELP,QUITPROG

'*****************************************************************************
'GENERATE A RAMP
'*****************************************************************************

GENERATE:
LOCATE 2,1
PRINT"                                                  "
LOCATE 3,1
PRINT"DEFAULT VALUE"
LOCATE 3,70
PRINT"NEW VALUE"
INPUTUNIDIRECTIONAL:
LOCATE 5,7
PRINT UNIPOLAR$
LOCATE 5,15
PRINT"                                  "
LOCATE 5,15
INPUT"Unipolar ramp ? (Y/N)         (Press RETURN for default) ",TEMP$

IF TEMP$ = "" THEN
    UNIPOLAR$ = UNIPOLAR$
```

```
ELSE
        UNIPOLAR$ = TEMP$
END IF

IF UNIPOLAR$ < > "Y" AND  UNIPOLAR$ < > "N" THEN GOTO INPUTUNIDIRECTIONAL

INPUTSTEPS:
LOCATE 7,5
PRINT USING "###";STEPS
LOCATE 7,15
PRINT"                                                  "
LOCATE 7,15
INPUT"Number of steps ?         (0 to skip, RETURN for default)  ",TEMP$

IF TEMP$ = "" THEN
        GOTO INPUTARC
ELSE
        STEPS = INT( VAL( TEMP$ ))
END IF

INPUTARC:
LOCATE 9,5
PRINT USING "###.#";ARC

LOCATE 9,15
PRINT"                                                  "
LOCATE 9,15
INPUT"Arc in degrees, for 1 step ? (0 to skip, RETURN for default)  ",TEMP$

IF TEMP$ = "" THEN
        ARC = ARC
ELSE
        ARC = VAL( TEMP$ )
END IF

IF STEPS * ARC > MAXARC THEN
        LOCATE 11,15
        PRINT"                                                  "
        LOCATE 11,15
        PRINT"Maximum size of arc for this number of steps is ";
        PRINT USING "###.###"; MAXARC/STEPS
        GOTO INPUTARC
ELSEIF ARC = 0 AND STEPS = 0 THEN
        LOCATE 11,15
        PRINT"                                                  "
        LOCATE 11,15
        PRINT"You must enter one of STEPS or ARC."
        GOTO INPUTSTEPS
END IF

INPUTRAMPDURATION:
LOCATE 11,1
PRINT USING "##.##";RAMPDURATION;
PRINT" ";
PRINT USING "###.##";VELOCITY
LOCATE 11,15
PRINT"                                                  "
LOCATE 11,15
INPUT"Ramp duration in seconds ?  (0 to skip, RETURN for default)  ",TEMP$

IF TEMP$ = "" THEN
        RAMPDURATION = RAMPDURATION
ELSE
        RAMPDURATION = VAL( TEMP$ )
END IF

IF RAMPDURATION * 2 * STEPS > MAXTOTALTIME AND UNIPOLAR$ = "N" THEN
        LOCATE 13,15
        PRINT"The maximum ramp duration for this number of steps is ";
```

```
        PRINT USING "###.###";MAXTOTALTIME / (2 * STEPS)
        GOTO INPUTRAMPDURATION
ELSEIF RAMPDURATION * STEPS > MAXTOTALTIME AND UNIPOLAR$ = "Y" THEN
        LOCATE 13,15
        PRINT"                                                  "
LOCATE 13,15
        PRINT"The maximum ramp duration for this number of steps is ";
        PRINT USING "##.###";MAXTOTALTIME / STEPS
        GOTO INPUTRAMPDURATION
ELSEIF RAMPDURATION = 0 THEN
        INPUTVELOCITY:
        LOCATE 11,1
        PRINT USING "##.##";RAMPDURATION;
        PRINT" ";
        PRINT USING "###.##";VELOCITY
        LOCATE 11,15
        PRINT"                                                  "
        LOCATE 11,15
        INPUT"Rate of movement in °/s     (0 to skip, RETURN for default)  ",TEMP$

        IF TEMP$ = "" THEN
                VELOCITY = VELOCITY
        ELSE
                VELOCITY = VAL( TEMP$ )
        END IF

        IF VELOCITY > MAXVELOCITY THEN
                LOCATE 13,15
                PRINT"The maximum velocity permitted is ";MAXVELOCITY
                GOTO INPUTVELOCITY
        ELSEIF VELOCITY = 0 THEN
                LOCATE 13,15
                PRINT"You must enter one of RAMP DURATION or VELOCITY"
                GOTO INPUTRAMPDURATION
        ELSEIF STEPS * ARC / VELOCITY > MAXTOTALTIME AND UNIPOLAR$ = "Y" THEN
                LOCATE 13,15
                PRINT"The minimum velocity for this arc and steps is ";
                PRINT USING "###.####";STEPS * ARC / MAXTOTALTIME
                GOTO INPUTVELOCITY
        ELSEIF 2 * STEPS * ARC / VELOCITY > MAXTOTALTIME AND UNIPOLAR$ = "N" THEN
                LOCATE 13,15
                PRINT"The minimum velocity for this arc and steps is ";
                PRINT USING "###.####";2 * STEPS * ARC / MAXTOTALTIME
                GOTO INPUTVELOCITY
        END IF

END IF

INPUTSTARTANGLE:
LOCATE 13,5
PRINT USING "###";STARTANGLE
LOCATE 13,15
PRINT"                                                  "
LOCATE 13,15
PRINT"Enter the start angle (";MINANGLE;" - ";MAXANGLE;"° )  (RETURN for default)  ";
INPUT, TEMP$
IF TEMP$ = "" THEN
        STARTANGLE = STARTANGLE
ELSE
        STARTANGLE = VAL( TEMP$ )
END IF

IF STARTANGLE > MAXANGLE OR STARTANGLE < MINANGLE THEN
        LOCATE 15,15
        PRINT"The value must lie within the limits given."
        GOTO INPUTSTARTANGLE
END IF

INPUTENDANGLE:
```

```
LOCATE 15,5
PRINT USING "###";ENDANGLE
LOCATE 15,15
PRINT"                                                  "
LOCATE 15,15
PRINT"Enter the peak angle  (";MINANGLE;" - ";MAXANGLE;"° )  (RETURN for default)  ";
INPUT, TEMP$
IF TEMP$ = "" THEN
        ENDANGLE = ENDANGLE
ELSE
        ENDANGLE = VAL( TEMP$ )
END IF

LOCATE 17,1
PRINT"                                                  "

                        'In a bipolar mountain, the ending angle = STARTANGLE.  ENDANGLE is the
                        'position of the peak or trough of the mountain (i.e. the halfway value)

IF ENDANGLE > MAXANGLE OR ENDANGLE < MINANGLE THEN
        LOCATE 17,15
        PRINT"The value must lie within the limits given."
        GOTO INPUTENDANGLE
END IF


'*****************************************************************************************
'CALCULATE AND CHECK RAMP ARC, STEPS, AND BOUNDS, THEN ASK FOR HOLD DURATION
'*****************************************************************************************

MOVEANGLE = ABS( STARTANGLE - ENDANGLE )

IF STEPS = 0 THEN
        STEPS = INT( MOVEANGLE / ARC )

        IF STEPS = 0 THEN
                LOCATE 17,15
                PRINT"You have made END - START less than ARC"
                GOTO INPUTSTARTANGLE
        END IF

ELSEIF STEPS * ARC > MOVEANGLE THEN
        TESTBOUNDS:
        LOCATE 17,5
        PRINT"(STEPS * ARC) is too large for these bounds.  (Change S, A, or B ?)";
        OVERRIDE$ = INPUT$(1)
        LOCATE 17,5
        PRINT"                                                  "

        IF OVERRIDE$ = "S" THEN

                IF ARC > MOVEANGLE THEN
                        LOCATE 17,15
                        PRINT"ARC is larger than BOUNDS so you can't just reduce STEPS."
                        GOTO TESTBOUNDS
                END IF

                STEPSOVERRIDE:
                LOCATE 17,15
                 PRINT"Set new value for STEP SIZE (1 - ";INT( MOVEANGLE/ARC);" )";
                INPUT STEPS
                STEPS = INT( STEPS )

                IF STEPS > INT( MOVEANGLE/ARC ) OR STEPS < 1 THEN
                        LOCATE 19,15
                        PRINT"Your value exceeded the limits stated."
                        GOTO STEPSOVERRIDE
                END IF
```

```
        ELSEIF OVERRIDE$ = "A" THEN
                ARCOVERRIDE:
                LOCATE 17,15
                PRINT"Set new value for ARC.  ( 0 - ";MOVEANGLE/STEPS;" )";
                INPUT ARC

                IF ARC > MOVEANGLE/STEPS OR ARC < 0 THEN
                LOCATE 19,15
                PRINT"Your value exceded the limits stated"
                GOTO ARCOVERRIDE
                END IF

        ELSEIF OVERRIDE$ = "B" THEN
                GOTO INPUTSTARTANGLE

        ELSE
                LOCATE 19,15
                PRINT"Enter S to change STEPS, A to change ARC, or B to change BOUNDS"
                GOTO TESTBOUNDS
        END IF

END IF

IF ARC = 0 THEN
        ARC = MOVEANGLE / STEPS
        LOCATE 19,15
        PRINT"                                                  "
        LOCATE 19,15
        PRINT"The calculated ARC is          ";
        PRINT USING "###.##";ARC;
        PRINT"°.             "
ELSE
        LOCATE 19,15
        PRINT"                                                  "
        LOCATE 19,15
        PRINT"The calculated number of STEPS is ";
        PRINT USING "###";STEPS;
        PRINT".                  "

END IF

IF RAMPDURATION = 0 THEN
        RAMPDURATION =  ARC / VELOCITY
        LOCATE 20,15
        PRINT"                                                  "
        LOCATE 20,15
        PRINT"The calculated RAMP DURATION is  ";
        PRINT USING "##.##";RAMPDURATION;
        PRINT"  seconds."
ELSE
        VELOCITY = ARC / RAMPDURATION
        LOCATE 20,15
        PRINT"                                                  "
        LOCATE 20,15
        PRINT"The calculated VELOCITY is       ";
        PRINT USING "###.####"; VELOCITY;
   P    RINT" °/s."
END IF

IF STEPS * ARC / VELOCITY > MAXTOTALTIME AND UNIPOLAR$ = "Y" THEN
        LOCATE 21,15
        PRINT"(STEPS * ARC / VELOCITY) exceeds MAX TIME."
        GOTO GENERATE
ELSEIF 2 * STEPS * ARC / VELOCITY > MAXTOTALTIME AND UNIPOLAR$ = "N" THEN
        LOCATE 21,15
        PRINT"(2 * STEPS * ARC / VELOCITY) exceeds the MAXIMUM TIME LIMIT.  "
        GOTO GENERATE
```

```
END IF

INPUTHOLDDURATION:
LOCATE 22,5
PRINT USING "##.##";HOLDDURATION
LOCATE 22,15
PRINT"                                                    "

LOCATE 22,15
IF UNIPOLAR$ = "Y" THEN
        MAXHOLDDURATION = (MAXTOTALTIME - STEPS * RAMPDURATION) / STEPS
        PRINT"Enter the HOLD duration ( 0 - ";
        PRINT USING "##.##"; MAXHOLDDURATION;
        PRINT "s). (RETURN for default) ";
        INPUT ,TEMP$

        IF TEMP$ = "" THEN
                HOLDDURATION = HOLDDURATION
        ELSE
                HOLDDURATION = VAL( TEMP$ )
        END IF

        LOCATE 23,1
        PRINT"                                                    "

        IF HOLDDURATION > MAXHOLDDURATION OR HOLDDURATION < 0 THEN
                LOCATE 23,15
                PRINT"The hold duration cannot exceed the limits shown."
                GOTO INPUTHOLDDURATION
        END IF

ELSE
        MAXHOLDDURATION = (MAXTOTALTIME - 2 * STEPS * RAMPDURATION) / (STEPS * 2)
        PRINT"Enter the HOLD DURATION ( 0 - ";
        PRINT USING "##.##"; MAXHOLDDURATION;
        PRINT ") sec. (RETURN for default) ";
        INPUT ,TEMP$

        IF TEMP$ = "" THEN
                HOLDDURATION = HOLDDURATION
        ELSE
                HOLDDURATION = VAL( TEMP$ )
        END IF

        IF HOLDDURATION > MAXHOLDDURATION OR HOLDDURATION < 0 THEN
                LOCATE 23,15
                PRINT"The hold duration cannot exceed the limits shown."
                GOTO INPUTHOLDDURATION
        END IF

END IF

RANGESELECT = MAXANGLE / VELOCITY

IF RANGESELECT > 250 THEN
        LOCATE 22,15
        PRINT"You can't set this velocity on the Ramp generator. Press a key   "
        DUMMY$ = INPUT$(1)
        GOTO GENERATE
ELSEIF RANGESELECT > = 100 THEN
        RANGEVAR = RANGESELECT / 100
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 100 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 50 THEN
        RANGEVAR = RANGESELECT / 50
        LOCATE1,15
        PRINT"Set RANGE SELECT to 50 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
```

```
ELSEIF RANGESELECT > = 20 THEN
        RANGEVAR = RANGESELECT / 20
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 20 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 10 THEN
        RANGEVAR = RANGESELECT / 10
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 10 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 5 THEN
        RANGEVAR = RANGESELECT / 5
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 5 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 2 THEN
        RANGEVAR = RANGESELECT / 2
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 2 and RANGE VARIABLE to ";
         PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 1 THEN
        RANGEVAR = RANGESELECT / 1
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 1 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 0.5 THEN
        RANGEVAR = RANGESELECT / 0.5
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 0.5 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 0.2 THEN
        RANGEVAR = RANGESELECT / 0.2
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 0.2 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSEIF RANGESELECT > = 0.11 THEN
        RANGEVAR = RANGESELECT / 0.11
        LOCATE 1,15
        PRINT"Set RANGE SELECT to 0.11 and RANGE VARIABLE to ";
        PRINT USING "#.###";RANGEVAR
ELSE
        LOCATE 22,15
        PRINT"You can't set this velocity on the ramp generator. Press a key   "
        DUMMY$ = INPUT$(1)
        GOTO GENERATE
END IF

IF ENDANGLE > STARTANGLE THEN
        STARTPOLARITY$ = "POS"
ELSE
        STARTPOLARITY$ = "NEG"
END IF

LASTCHANCE:
CHECKEDFLAG$ = "YES"  ' ALLOWS VALUES TO BE SAVED TO DISK
LOCATE 2,1
PRINT"                                                    "
LOCATE 3,1
PRINT"                                                    "
LOCATE 2,5
PRINT"Are these values correct and is the ramp generator set (Y/N) or Q to quit"
TEMP$ = INPUT$(1)
LOCATE 1,1
PRINT"                                                    "
LOCATE 2,1
PRINT"                                                    "
IF TEMP$ < > "Y" AND TEMP$ < > "Q" THEN GOTO GENERATE
IF TEMP$ = "Q" THEN GOTO FIRSTMENU
```

```
RAMPOUTPUT:
IF CURRENTANGLE < > STARTANGLE THEN GOSUB SETUPSTART

TEMPRAMPDURATION = RAMPDURATION * 1000                    ' CONVERTS TO MILLISECONDS

IF TEMPRAMPDURATION < 300 THEN
        COUNT# = -0.84952 + 0.3150055 * TEMPRAMPDURATION  ' TIMING LOOP DIFFERENT
ELSE                                                      ' FOR DIFFERENT
        COUNT# = -2.85672 + 0.3179169 * TEMPRAMPDURATION  ' DURATIONS
END IF

TEMP = INT (COUNT#)

IF COUNT# - TEMP > = 0.5 THEN
        COUNT# = INT( COUNT# + 1 )                                ' ROUNDING UP
ELSE
        COUNT# = INT( COUNT# )
END IF

LOCATE 2,1
PRINT"      Now outputting to the RS-232 serial port using the DTR & RTS lines.  "

'*****************************************************************************************
'USE THE INPUT VALUES TO TIME THE OUTPUT OF PULSES IN THE RS232 PORT
'*****************************************************************************************

IF UNIPOLAR$ = "Y" AND STARTPOLARITY$ = "POS" THEN
        GOSUB POSRAMP
        CURRENTANGLE = CURRENTANGLE + VELOCITY * RAMPDURATION * STEPS
ELSEIF UNIPOLAR$ = "Y" AND STARTPOLARITY$ = "NEG" THEN
        GOSUB NEGRAMP
        CURRENTANGLE = CURRENTANGLE - VELOCITY * RAMPDURATION * STEPS
ELSEIF UNIPOLAR$ = "N" AND STARTPOLARITY$ = "POS" THEN
        GOSUB POSRAMP
        GOSUB NEGRAMP
ELSE
        GOSUB NEGRAMP
        GOSUB POSRAMP
END IF

'*****************************************************************************************

REPEAT:
LOCATE 2,1
PRINT"                                                  "
LOCATE 2,5
PRINT"Quit (Q), Add to (A), exactly repeat (E), reverse (R) or modify (M) ?"
REPEAT$ = INPUT$(1)

IF REPEAT$ < > "Q" AND REPEAT$ < > "A" AND REPEAT$ < > "E" AND REPEAT$ < > "R" AND REPEAT$
< > " " AND REPEAT$ < > "M" THEN
        GOTO REPEAT
ELSEIF REPEAT$ = "Q" THEN
        GOTO FIRSTMENU
ELSEIF REPEAT$ = "M" THEN
        GOTO GENERATE
ELSEIF UNIPOLAR$ = "N" AND REPEAT$ < > "E" AND REPEAT$ < > " " THEN
        LOCATE 2,1
        PRINT"Bipolar ramps can only be repeated.  Press a key to repeat ramp (Q to escape)."
        DUMMY$ = INPUT$(1)
        IF DUMMY$ = "Q" THEN GOTO FIRSTMENU
        GOTO RAMPOUTPUT
ELSEIF UNIPOLAR$ = "N" THEN
        GOTO RAMPOUTPUT
ELSEIF TRUNCATEDFLAG$ = "YES" THEN
        LOCATE 2,1
        PRINT"                                                  "
        LOCATE 2,18
        PRINT"You cannot rerun this ramp.  Press RETURN to continue.";
```

```
        INPUT,DUMMY$
        TRUNCATEDFLAG$ = "NO"
        GOTO FIRSTMENU
ELSEIF REPEAT$ = "E" OR REPEAT$ = " " THEN
        GOTO RAMPOUTPUT
ELSEIF REPEAT$ = "A" THEN

        IF CURRENTANGLE + VELOCITY * RAMPDURATION * STEPS > ENDANGLE AND
STARTPOLARITY$ = "POS"
        OR CURRENTANGLE - VELOCITY * RAMPDURATION * STEPS < ENDANGLE AND
STARTPOLARITY$ = "NEG" THEN
                CHECKEDFLAG$ = "NO"                     ' DISALLOWS VALUES TO BE SAVED
                TRUNCATED:
                LOCATE 2,1
                PRINT"                                  "
                LOCATE 2,5
                PRINT"This additive ramp will be shorter so that ENDANGLE is not exceeded."
                LOCATE 3,1
                PRINT"                                  "
                LOCATE 3,12
                PRINT"Type Y to continue, or N to return to previous options."
                DUMMY$ = INPUT$(1)

                IF DUMMY$ < > "Y" AND DUMMY$ < > "N" THEN
                        LOCATE 3,1
                        PRINT"                                  "
                        GOTO TRUNCATED
                ELSEIF DUMMY$ = "N" THEN
                        LOCATE 3,1
                        PRINT"                                  "
                        GOTO REPEAT
                ELSE
                        TRUNCATEDFLAG$ = "YES"
                        REDUCTION:
                        LOCATE 3,1
                        PRINT"                                  "
                        LOCATE 2,1
                        PRINT"                                  "
                        LOCATE 2,16
                        PRINT"Do you want to reduce STEPS (S) or RAMP DURATION (R)"
                        DUMMY$ = INPUT$(1)

                IF DUMMY$ < > "S" AND DUMMY$ < > "R" THEN
                        GOTO REDUCTION
                ELSEIF DUMMY$ = "R" THEN
                        RAMPDURATION = ABS( ENDANGLE - CURRENTANGLE ) / ( VELOCITY * STEPS )
                        STARTANGLE = CURRENTANGLE
                        GOTO RAMPOUTPUT
                ELSE
                        STEPS = INT(ABS(ENDANGLE-CURRENTANGLE)/(RAMPDURATION*VELOCITY))
                        STARTANGLE = CURRENTANGLE
                        GOTO RAMPOUTPUT
                END IF

        END IF

        ELSE
                STARTANGLE = CURRENTANGLE
                GOTO RAMPOUTPUT
        END IF

ELSEIF STARTPOLARITY$ = "POS" THEN
        STARTPOLARITY$ = "NEG"
        TEMPANGLE = STARTANGLE                  ' REVERSES THE START AND END ANGLES
        STARTANGLE = CURRENTANGLE       ' BUT NOTE THAT ENDANGLE IS NOW
        ENDANGLE = TEMPANGLE            ' REDUCED TO THE CURRENT FINAL ANGLE
        GOTO RAMPOUTPUT
ELSEIF STARTPOLARITY$ = "NEG" THEN
        STARTPOLARITY$ = "POS"
```

```
        TEMPANGLE = STARTANGLE              ' REVERSES THE START AND END ANGLES
        STARTANGLE = CURRENTANGLE  ' BUT NOTE THAT ENDANGLE IS NOW
        ENDANGLE = TEMPANGLE          ' REDUCED TO THE CURRENT FINAL ANGLE
        GOTO RAMPOUTPUT

END IF

'*****************************************************************************************

THEEND:
END

'*****************************************************************************************
'SAVE PARAMETERS TO DISK
'*****************************************************************************************

SAVEPARAM:

IF CHECKEDFLAG$ = "NO" THEN
        CLS
        LOCATE 6,13
        PRINT"You must enter a complete set of values before saving."
        LOCATE 8,9
        PRINT"Either run a ramp and then SAVE, or enter values and exit using"
        LOCATE 10,14
        PRINT"the Q option when asked if all the values are correct."
        LOCATE 16,27
        PRINT"Press RETURN to continue";
        INPUT, DUMMY$
        GOTO FIRSTMENU
END IF
CLS

LOCATE 7,16
PRINT"Do you want a list of the files on disk ? (Y/N)  "
DUMMY$ = INPUT$(1)

IF DUMMY$ = "N" THEN GOTO DESTINATION

GOSUB FILEDIRECTORY

DESTINATION:
LOCATE 16,1
PRINT"                                        "
LOCATE 17,1
PRINT"                                        "
LOCATE 14,9
PRINT"Enter drive and filename to use ( E.g. B:\TESTPROG ) or Q to QUIT. "
LOCATE 16,31
PRINT"_________.RMP"
DUMMY$ = INPUT$(1)

IF DUMMY$ = "Q" THEN
        GOTO FIRSTMENU
ELSE
        LOCATE 16,31
        PRINT DUMMY$;
        INPUT, FILENAME$
        FILENAME$ = DUMMY$ + FILENAME$
END IF

IF LEFT$(FILENAME$,3) < > "A:\" AND LEFT$(FILENAME$,3) < > "B:\" THEN
        LOCATE 18,5
        PRINT"You must enter the drive name at the start. Use either A:\.... or B:\...."
        GOTO DESTINATION
END IF

FILENAME$ = LEFT$(FILENAME$,11)
```

```
IF LEN( MID$( FILENAME$,4,8 )) = 0 OR MID$( FILENAME$,4,1 ) = " " THEN
        LOCATE 18,1
        PRINT"                                                    "
        LOCATE 18,6
        PRINT"You must enter a valid file name following the drive specifier."
        LOCATE 20,6
        PRINT"You may use up to 8 characters or numerals.  Don't use spaces."
        LOCATE 23,25
        INPUT"Press RETURN to continue",DUMMY$
        LOCATE 18,6
        PRINT"                                                    "
        LOCATE 20,6
        PRINT"                                                    "
        LOCATE 23,20
        PRINT"                    "
        GOTO DESTINATION
END IF

FILENAME$ = LEFT$(FILENAME$,11) + ".RMP"
LOCATE 20,35
PRINT"Saving..."
OPEN FILENAME$ FOR OUTPUT AS #1
WRITE                                                                                    #1
,UNIPOLAR$,STEPS,ARC,RAMPDURATION,VELOCITY,STARTANGLE,ENDANGLE,HOLDDURATION
CLOSE #1
LOCATE 22,25
PRINT"File written as ";FILENAME$;"."
LOCATE 24,27
PRINT"Press RETURN to continue.";
INPUT, DUMMY$
GOTO FIRSTMENU

'*****************************************************************************************
'READ PARAMETERS FROM DISK
'*****************************************************************************************
READPARAM:
LOCATE 7,16
PRINT"Do you want a list of the files on disk ? (Y/N)  "
DUMMY$ = INPUT$(1)

IF DUMMY$ = "N" THEN GOTO SOURCE

GOSUB FILEDIRECTORY

SOURCE:
LOCATE 16,1
PRINT"                                    "
LOCATE 17,1
PRINT"                                    "
LOCATE 14,9
PRINT"Enter drive and filename to use ( E.g. B:\TESTPROG ) or Q to QUIT."
LOCATE 16,31
PRINT"_________.RMP"
DUMMY$ = INPUT$(1)

IF DUMMY$ = "Q" THEN
        GOTO FIRSTMENU
ELSE
        LOCATE 16,31
        PRINT DUMMY$;
        INPUT, FILENAME$
        FILENAME$ = DUMMY$ + FILENAME$
END IF

IF LEFT$(FILENAME$,3) < > "A:\" AND LEFT$(FILENAME$,3) < > "B:\" THEN
        LOCATE 18,5
        PRINT"You must enter the drive name at the start. Use either A:\.... or B:\...."
        GOTO SOURCE
END IF
```

```
FILENAME$ = LEFT$(FILENAME$,11)

IF LEN( MID$( FILENAME$,4,8 )) = 0 OR MID$( FILENAME$,4,1 ) = " " THEN
        LOCATE 18,1
        PRINT"                                                  "
        LOCATE 18,6
        PRINT"You must enter a valid file name following the drive specifier."
        LOCATE 20,6
        PRINT"You may use up to 8 characters or numerals.  Don't use spaces."
        LOCATE 23,25
        INPUT"Press RETURN to continue",DUMMY$
        LOCATE 18,6
        PRINT"                                          "
        LOCATE 20,6
        PRINT"                                          "
        LOCATE 23,20
        PRINT"                    "
        GOTO SOURCE
END IF

FILENAME$ = LEFT$(FILENAME$,11) + ".RMP"
LOCATE 20,35
PRINT"Reading..."
OPEN FILENAME$ FOR INPUT AS #1
INPUT# 1, UNIPOLAR$, STEPS, ARC, RAMPDURATION, VELOCITY, STARTANGLE, ENDANGLE,
HOLDDURATION
CLOSE #1
LOCATE 22,20
PRINT"File ";FILENAME$;" was read successfully."
LOCATE 24,27
PRINT"Press RETURN to continue.";
INPUT, DUMMY$
GOTO FIRSTMENU

'*****************************************************************************************
'QUIT THE PROGRAM
'*****************************************************************************************

QUITPROG:

CLS
LOCATE 12,11
PRINT"You have asked to QUIT this program. Is this correct (Y/N)"
TEMP$ = INPUT$(1)

IF TEMP$ < > "Y" THEN GOTO FIRSTMENU

CLS
GOTO THEEND
RETURN

'*****************************************************************************************
'DEFINE SUBROUTINES
'*****************************************************************************************

MANUALRESET:    'RESETS CURRENTANGLE TO ZERO.  ASSUMES THAT RAMP GEN SET TO 0

CLS
LOCATE 12,1
PRINT"You MUST reset the ramp generator to 0 volts (60°) using its appropriate switch."
LOCATE 14,4
PRINT"Press Y when done, or N to escape without resetting computer or generator."
RESETTOZERO$ = INPUT$(1)

IF RESETTOZERO$ < > "Y" AND RESETTOZERO$ < > "N" THEN
        GOTO MANUALRESET
ELSEIF RESETTOZERO$ = "N" THEN
        GOTO FIRSTMENU
```

```
ELSE
        CHECKEDFLAG$ = "NO"
        CURRENTANGLE = 60
        LOCATE 16,16
        PRINT"The entire system is now reset to 0 volts =";CURRENTANGLE;"°"
        LOCATE 20,27
        INPUT"Press RETURN to continue",DUMMY$
END IF

GOTO FIRSTMENU

'*****************************************************************************

SETUPSTART:                                     'SETS CURRENTANGLE TO STARTANGLE

IF STARTANGLE > CURRENTANGLE THEN
        POLARITY$ = "POS"
ELSE
        POLARITY$ = "NEG"
END IF

DURATION = ABS(STARTANGLE - CURRENTANGLE) / VELOCITY
        LOCATE 2,1
        PRINT"                                          "
        LOCATE 2,17
        PRINT"It will take";
        PRINT USING "###.#";DURATION;
        PRINT" seconds to reset the start angle."

TEMPDURATION = DURATION * 1000                  ' CONVERTS TO MILLISECONDS FOR TIMING

IF TEMPDURATION < 300 THEN
        COUNT# = -0.84952 + 0.3150055 * TEMPDURATION ' DIFFERENT TIMING
ELSE                                            ' FOR DIFFERENT
        COUNT# = -2.85672 + 0.3179169 * TEMPDURATION ' DURATIONS
END IF

TEMP = INT (COUNT#)

IF COUNT# - TEMP > 0.5 THEN                     ' ROUNDING UP OR DOWN
        COUNT# = INT( COUNT# + 1)
ELSE
        COUNT# = INT( COUNT# )
END IF

IF POLARITY$ = "POS" THEN
        OUT(PORT), 0                            ' RESET LINES
        OUT(PORT), 1                            ' RAMP ON
        FOR COUNTER = 1 TO COUNT#               ' USE LOOP TO TIME DELAY
        NEXT COUNTER
        OUT(PORT), 0                            ' RAMP OFF
        LOCATE 2,1
        PRINT"                                  "
        LOCATE 2,17
        PRINT"Waiting for";SETTLINGTIME;" seconds before outputting."
        DELAY SETTLINGTIME                      ' THIS TIME IS NOT CRITICAL SO JUST USE DELAY
ELSE
        OUT(PORT), 2                            ' RESET LINES
        OUT(PORT), 3                            ' RAMP ON
        FOR COUNTER = 1 TO COUNT#                       ' USE LOOP TO TIMEE DELAY
        NEXT COUNTER
        OUT(PORT), 2                            ' RAMP OFF
        LOCATE 2,1
        PRINT"                                  "
        LOCATE 2,12
        PRINT"Waiting for";SETTLINGTIME;" seconds before outputting ramp."
        DELAY SETTLINGTIME                      ' THIS TIME IS NOT CRITICAL SO JUST USE DELAY
END IF
```

```
CURRENTANGLE = STARTANGLE

RETURN

'*****************************************************************************************

POSRAMP:                                        ' MAKES A POSITIVE RAMP USING DTR LINE

OUT(PORT), 0                                    'RESET LINES
FOR STAIRCASE = 1 TO STEPS
        DELAY HOLDDURATION                  ' ACCURACY APPROX 0.054 SEC - NOT CRITICAL
        OUT(PORT), 1                            ' RAMP ON
        FOR COUNTER = 1 TO COUNT#               ' USE LOOP TO TIME RAMP ACCURATELY
        NEXT COUNTER
        OUT(PORT), 0                            ' RAMP OFF
NEXT STAIRCASE

RETURN

'*****************************************************************************************

NEGRAMP:

OUT(PORT), 2                                    'RESET LINES
FOR STAIRCASE = 1 TO STEPS
        DELAY HOLDDURATION                  ' ACCURACY APPROX 0.054 SEC - NOT CRITICAL
        OUT(PORT), 3                            ' RAMP ON
        FOR COUNTER = 1 TO COUNT#               ' USE LOOP TO TIME RAMP ACCURATELY
        NEXT COUNTER
        OUT(PORT), 2                            ' RAMP OFF
NEXT STAIRCASE

RETURN

'*****************************************************************************************

FILEDIRECTORY:

CLS
LOCATE 1,1
PRINT"  Enter the DRIVE or MASK (if desired), or press RETURN for A:\*.RMP"
LOCATE 2,35
INPUT,DUMMY$

IF DUMMY$ = "" THEN DUMMY$ = "A:\*.RMP"

LOCATE 2,32
PRINT"                  "
LOCATE 1,1
PRINT"                                                  "
LOCATE 1,1
PRINT"Directory of ";DUMMY$
FILES DUMMY$

RETURN

'*****************************************************************************************

HELP:

CLS
LOCATE 1,22
PRINT"Page 1 of 5.  Press a key to continue."
LOCATE 3,22
PRINT"    GENERAL INFORMATION"
LOCATE 5,1
PRINT"This program is designed to control an analogue ramp generator (R.G.) via"
PRINT"an RS-232 serial port on an IBM  PC compatible computer.  The program can"
PRINT"turn the ramp on or off, and control the polarity of the ramp.  The program"
```

```
PRINT"assumes that the leg was set to 60° (0 volts) when the forceps were attached."
PRINT
PRINT"The slope (velocity) of the ramp is set by a potentiometer on the front"
PRINT"panel of the R.G. but the program must be told this value prior to"
PRINT"outputting the ramp. The program allows for input of a variety of parameters"
PRINT"including ramp duration, hold duration, and number of steps. Values not"
PRINT"input are calculated by the program where necessary. If the number of steps"
PRINT"is not input, then the program will do as many as allowed by the current"
PRINT"settings of the start and end angles, and the ramp arc. They will all be"
PRINT"the same size. If you ask to skip the ramp duration option then the program"
PRINT"asks for the velocity."
PRINT"In this way a staircase ramp function with many steps can be easily and"
PRINT"repeatably created. Staircases may be unidirectional or bidirectional, in"
PRINT"which case the voltage increases or decreases then returns in an identical way"
PRINT"to the starting voltage. Using only 1 step results in the production of"
PRINT"a linear ramp between the start and end angles."

DUMMY$ = INPUT$(1)
CLS
LOCATE 1,22
PRINT"Page 2 of 5. Press a key to continue."
LOCATE 3,22
PRINT"      GENERAL INFORMATION"
LOCATE 5,1
PRINT"The starting angle can be set to any realistic value, and the initial slope"
PRINT"can be positive or negative. The program tests for a number of input errors"
PRINT"and allows for their alteration. Ramps may be repeated and reversed as long"
PRINT"the bounds are not exceeded."
PRINT
PRINT"There are a number of constants set out at the start of the program"
PRINT"listing. These provide limits for the ramp velocity and total duration,"
PRINT"as well as the arc of movement and extremes of angle. If you want to"
PRINT"change these, you will need to modify the source coding in TURBO BASIC."
PRINT
PRINT"There is facility to save a set of values to disk for later recall."
PRINT"Before saving, you must enter a valid set of parameters in the GENERATE"
PRINT"option. You may run the ramp and then SAVE, or exit to the main menu and"
PRINT"SAVE immediately. A directory of the files on the disk can be obtained"
PRINT"from either the SAVE or READ options."

DUMMY$ = INPUT$(1)
CLS
LOCATE 1,22
PRINT"Page 3 of 5. Press a key to continue."
LOCATE 3,22
PRINT"      GENERAL INFORMATION"
LOCATE 5,1
PRINT"When reading a set of parameters from disk, the current values are erased."
PRINT"Values are read in, and then you are taken to the GENERATE option, where"
PRINT"you have the option of modifying any value. The loaded values are displayed"
PRINT"down the left side of the screen. You must verify each value by pressing"
PRINT"RETURN, or entering a new value. This provides a check in case the constants"
PRINT"have changed since the parameters were stored."
PRINT
PRINT"There is facility to manually reset the entire program and R.G. to zero volts."
PRINT"This is a safety feature provided in the case that you think that the "
PRINT"program has lost track of the current angle. This may happen if the "
PRINT"velocity is entered incorrectly, or if the velocity potentiometer on the"
PRINT"front panel of the R.G. is set incorrectly. You must choose this option"
PRINT"then reset the R.G. using the reset switch provided on its front panel."
PRINT"The program will then reset the value of current angle to zero."

DUMMY$ = INPUT$(1)
CLS
LOCATE 1,22
PRINT"Page 4 of 5. Press a key to continue."
LOCATE 3,22
PRINT"      TECHNICAL INFORMATION"
LOCATE 5,1
```

```
PRINT"This program was written in TURBO BASIC and compiled to disk as an EXE file."
PRINT"If you want to make changes to the program, you must run TB and edit the"
PRINT"original source program. You can run this directly from TB for debugging"
PRINT"but it is should be compiled to disk eventually so that it can be run from"
PRINT"the DOS prompt. At the moment the program works fine - so please test any"
PRINT"modifications thoroughly before deleteing this version !! "
PRINT
PRINT"The program uses the DTR line of the serial port (COM1 or COM2) to switch"
PRINT"the ramp on or off. If DTR is high then the ramp is on. The RTS line is"
PRINT"used to control the polarity of the ramp. If RTS is high then the ramp is"
PRINT"negative-going, otherwise it is positive-going."
PRINT
PRINT"Both RTS and DTR can be switched on or off by writing different values to"
PRINT"the appropriate memory location (Hex 3FC for COM1 or Hex 2FC for COM2)."
PRINT
PRINT"          A value of 0 turns DTR off and RTS off"
PRINT"          A value of 1 turns DTR on  and RTS off"
PRINT"          A value of 2 turns DTR off and RTS on"
PRINT"          A value of 3 turns DTR on  and RTS on"

DUMMY$ = INPUT$(1)

CLS
LOCATE 1,22
PRINT"Page 5 of 5. Press a key to return to menu."
LOCATE 3,22
PRINT"    TECHNICAL INFORMATION"
LOCATE 5,1
PRINT"Timing of the hold durations is done using the DELAY function, which although"
PRINT"accurate to only 0.054 sec, is adequate for these non-critical times. The"
PRINT"durations of the ramps, and the initial setting of the start angle need to be"
PRINT"timed more accurately, and so a FOR/NEXT loop is used. The MTIMER function"
PRINT"seems to have a number of problems and inaccuracies, so it was not used. The"
PRINT"loop method works well, but is specific for a computer running at 4.77 MHz, so"
PRINT"IF YOU CHANGE MACHINES, CHECK THE TIMING-YOU MAY NEED TO ALTER THE
CALCULATIONS"
PRINT"The way to do this is to write the following small program to test the time"
PRINT"taken for loops with different COUNT#. I found that the regression through the"
PRINT"times was not linear, but that two linear fits split at the 300 millisec point"
PRINT"worked fine. PROGRAM:   MTIMER                                    "
PRINT"                COUNT# = 30        ' OR OTHER APPROPRIATE VALUES"
PRINT"                FOR TEMP = 1 TO COUNT#                    "
PRINT"                NEXT TEMP                          "
PRINT"                PRINT MTIMER                         "
PRINT"Plot the points over the ranges desired (e.g. 100 - 300 msec & > 300 msec) and"
PRINT"compute the regression equation which will calculate the size of COUNT# for any"
PRINT"given time. The shortest accurate duration is about 150 msec for a 4.77 MHz"
PRINT"computer. The timing calculations in the program will then need to be changed."

DUMMY$ = INPUT$(1)
GOTO FIRSTMENU

'*****************************************************************************
'DEFINE ERROR TRAPPING PROCEDURES
'*****************************************************************************

ERRORTRAP:

CLS
LOCATE 5,15
PRINT"There seems to be a problem with the last command."
' ERR RETURNS THE ERROR CODE
' ERDEV$ RETURNS THE DEVICE

IF ERR = 53 THEN
        LOCATE 10,1
        PRINT"This is not a valid path or filename, or there are no files matching this mask."
        LOCATE 12,1
        PRINT"      Recheck the path, use an existing filename, or change the mask."
```

```
ELSEIF ERR = 57 THEN
        LOCATE 10,10
        PRINT"There is a fault with ";ERDEV$
        LOCATE 12,10
        PRINT"Check this device before retrying."
        CLOSE
ELSEIF ERR = 61 THEN
        LOCATE 10,1
        PRINT" The disk is full.  You must delete some files before saving this file."
        LOCATE 12,1
        PRINT"You can't delete files from within this program so you must replace your"
        LOCATE 14,1
        PRINT"     disk with one with some space on it before retrying to SAVE."
        CLOSE
ELSEIF ERR = 68 THEN
        LOCATE 10,10
        PRINT ERDEV$;" is not available."
        LOCATE 12,10
        PRINT"Check this device before retrying."
        CLOSE
ELSEIF ERR = 70 THEN
        LOCATE 10,10
        PRINT"The disk in drive ";ERDEV$;" has a write protect tab on it."
        LOCATE 12,10
        PRINT"          Remove the tab before retrying."
        CLOSE
ELSEIF ERR = 71 THEN
        LOCATE 10,10
        PRINT"Drive ";ERDEV$;" door is open, or there is no disk in the drive."
        LOCATE 12,10
        PRINT"    Insert a disk and close the door before retrying."
        CLOSE
ELSEIF ERR = 72 THEN
        LOCATE 10,10
        PRINT" The disk in drive ";ERDEV$;"has a media error, it may be unusable."
        LOCATE 12,10
        PRINT"Retry the operation, but do not trust this disk with vital information."
        LOCATE 14,10
        PRINT"      Otherwise replace the disk with a good one and retry."
        CLOSE
ELSEIF ERR = 75 THEN
        LOCATE 10,10
        PRINT"You have made an error in the use of the path or file."
        LOCATE 12,10
        PRINT"  Check the syntax of the last command and retry."
        CLOSE
ELSEIF ERR = 76 THEN
        LOCATE 10,10
        PRINT"This path does not exist on the disk specified."
        LOCATE 12,10
        PRINT"Check the syntax of the last command and retry."
        CLOSE
ELSE
        LOCATE 10,10
        PRINT"       This is an unknown error."
        LOCATE 12,10
        PRINT"         The error code is";ERR
        LOCATE 14,10
        PRINT"      The device in error is ";ERDEV$
        LOCATE 16,10
        PRINT"You may need to quit the program and try again."
        CLOSE
END IF

LOCATE 20,26
PRINT"Press a key to continue"
DUMMY$ = INPUT$(1)
RESUME FIRSTMENU

'*****************************************************************************
'END OF LISTING
'*****************************************************************************
```

APPENDIX II

Miscellaneous techniques and trouble-shooting

SALINE

The following saline was used for all experiments. It kept for 2-3 weeks in the fridge before beginning to precipitate. The stock solutions kept for several months before precipitating. These solutions were checked visually before each batch of saline was made up. The saline was made with refrigerated distilled water (1 litre at a time). **Once all the chemicals and sucrose had been added, it was be aerated *before* the pH was adjusted.** In practice, I did not have to add NaOH to adjust the pH: it was correct anyway. Altering the length of time for aeration affected the pH. I used about 30 min.

Stock solutions

SOLUTION		QUANTITY		
	MW	g/l	g/500 ml	g/250 ml
2 M NaCl	58	116	58	29
1 M KCl	74.56	74.56	37.28	18.64
0.5 M $NaHCO_3$	84.01	42.01	21.01	10.5
1 M $NaH_2PO_4.2H_2O$	156.01	156.01	78.01	39
1 M $CaCl_2.6H_2O$	219.08	219.08	109.54	54.77
1 M NaOH	40	40	20	10

Saline

SOLUTION	QUANTITY		FINAL CONCENTRATION
	(5l)	(1l)	mM/l
2 M NaCl	350 ml	70 ml	140
1 M KCl	50 ml	10 ml	10
0.5 M $NaHCO_3$	40 ml	8 ml	4
1 M $NaH_2PO_4.2H_2O$	30 ml	6 ml	6
1 M $CaCl_2.6H_2O$	20 ml	4 ml	4

The correct volume of each of the first 4 solutions (in the order given) was added

to a flask containing approximately 90% of the total required volume of refrigerated distilled water. This was stirred while adding solutions to prevent local precipitation in regions of high chemical concentration. The $CaCl_2$ was added last. Sucrose was added at the rate of 34 $g.l^{-1}$ (it can be omitted, but I always used it). Finally, the rest of the distilled water was added.

The solution was aerated (NOT oxygenated) for approximately 30 min and adjusted to a pH of 6.8 with 1 M NaOH (generally not necessary).

MICROELECTRODES AND DYE INJECTION

Electrodes characteristically had resistances of 150-200 MOhm when filled with a saturated solution of hexamminecobalt (III) chloride (30-60 MOhm with 2 M KAc). SEM observation of the tip diameters of 6 randomly selected electrodes pulled in one session gave a mean outer diameter of 0.86 *u*m (range 0.45-1.8 *u*m). In order to achieve these high resistance electrodes, the following settings were used on the Narishige electrode puller. The values given here are the distances in millimetres from the centre of each solenoid roller to the bottom of the steel plate immediately above:

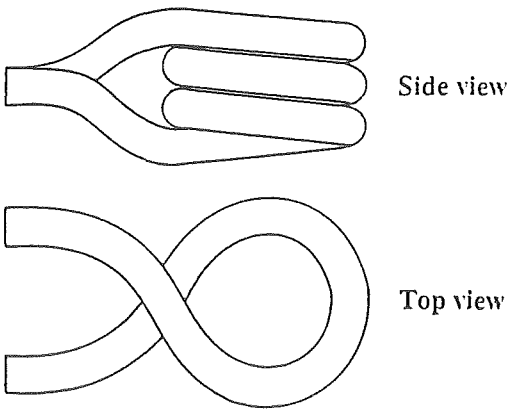
Left front solenoid: 40 mm.

Right front solenoid: 10 mm.

Rear solenoid: 8 mm.

Heater setting = 5.3, Magnet setting = 9.8: total current = 24 A.

The heater element (see illustration) was made from 1 mm nickel-chromium wire wound onto a suitable former. The inside diameter was 5 mm. The gap between subsequent turns was made as small as possible (a scalpel blade was used to separate touching coils).



The electrodes were pulled from Clark Electromedical Instruments capillary tubing GC100F-10 (with internal filament).

For most recordings Hexamminecobalt (III) Chloride was used. Five ml of saturated solution was made up at room temperature,

filtered (0.45 um millipore syringe filter), and diluted with a few drops of distilled water (to stop electrode blockage due to current-induced precipitation in the electrode). This solution did not seem to deteriorate, but every couple of weeks it was filtered as a precaution. This seemed to reduce electrode noise on occasions, and may have helped reduce electrode blockage. Injection current was not monitored (although ideally it would be), and generally, fills were satisfactory. The injection current polarity was reversed momentarily a few times every 5 min during injection to help minimise blockage of the electrode tip. I did not chloridise the earth or electrode silver wires, but I did clean them every few months with very fine sandpaper.

INTENSIFICATION

The method outlined below is a conglomerate based on several papers. It worked reasonably well for intensifying single cobalt-filled neurones within locust metathoracic ganglia.

1. Take specimen to water through an ethanol series (15 min in each of: 100%, 90%, 50%, and distilled water).
2. Leave 20 min in warm distilled water (44-48° oven).
3. Transfer to warm developer base for 90 min (in oven).
4. Transfer to developer (10:1 developer base: 1% $AgNO_3$).

Keep in oven, checking the development quickly under the microscope after the first 15 min, and then approximately every 5 min (or as needed, depending on the speed of the reaction, which is quite variable: experience will help here). Minimise the time out of the oven, because the reaction is light sensitive. If the intensification is not complete within 30 min then transfer to a new lot of developer. Generally this was not required.

Use glass instruments to handle the tissue. Keep the glassware clean. The pH should be between 2.5 and 3, but I generally did not check this. Some authors are very finicky about pH because it does affect development times, and can cause unacceptable amounts of precipitation onto the outer surface of the preparation. I found pH-paper too unreliable, and decided to just make up the solutions quite carefully instead. Once the hydroquinone has been added the developer base starts to deteriorate. After 4 days it was not usable. The base and silver nitrate were stored in the 45° oven.

Developer base

1.5 g powdered gum acacia (grind using mortar & pestle).
0.41 g citric acid.
5 g sucrose.

Grind the gum acacia in a mortar, then add the citric acid and sucrose, and stir. Make up to 80 ml with hot distilled water (70°), and stir well. Just prior to use add 0.085 g hydroquinone per 80 ml of base. Adjust pH to 2.6 with citric acid.

RECORDING PROBLEMS

The only major recording problem was caused by introducing foreign metals to the preparation (i.e., anything other that silver). Symptoms included increased electrode noise, large baseline shifts (often forcing the MEA-1 amplifier off to one or other extreme), and loss of frequency compensation. Altering the earthing did not help in any case. If saline came in contact with the side of the metal preparation dish this problem would occur even if the dish was well earthed. Therefore, it is necessary to use plasticine to ensure that the animal is completely isolated from the dish.

Initially there was a problem of very high frequency feedback in the vibrator/power amp/oscilloscope circuitry (at some 'leg angles' only). This was caused by a complex interaction of the wiring and the various devices. I ended up minimising the length of cable, and using silver-cored BNC cables. We also bridged the output of the power amp (= input to the vibrator) with a 104K 50, NIS T capacitor. This last step may be all that is necessary. Once set up, there were no further problems.

DATA SHEETS

Several forms based on the one illustrated overpage were designed on the computer and printed out, to make recording of experimental information easier. These forms were preferred to notebook recordings because missing information could easily be detected before the end of the experiment by the conspicuous blanks in the form.

Locusta migratoria F.C.O. response/morphology.
T. Matheson, Zoology Dept, Univ. of Canterbury.

Animal No. 125 Time 7.45am Dissection notes:
Date 24.8.90
Sex Male
Size Medium
Cuticle Firm
Haem. Some haemocytes
Fat Lots.

No problems. Animal ventilating strongly.
Ready to go @ 9.20am.

Tape No 19 . 4.8cm/s. 057 - 128

	Info.	Gain
CH 1	Ramp	2
CH 2	Stimulus	10
CH 3	FCO afferent	5
CH 4	Memo	

	FCO afferent
Dye	≈ Saturated Cobalt Hexammine
Res	>100 MΩ
R.pot	50mV
Spk	42mV → 48mV
Dam	No - improved during recording
Gain	X100

Notes
P=40 V⁻ A+- ← stronger at start of ramps.

FILL	
Begin	10.00am
Volts	2.5
Freq.	2.5Hz
Pulse	200ms
Length	30 min
Wait	30 min
Amp.	Fill

Notes
Axon visible when in ammonium sulfide.

	1	2	3	4
Arc	120°	20	20	20
Len.	0.2	0.05	0.5	0.1
Start	0	0	0	0
End	120	120	120	120
Hold	0	3	3	3
Ramp	600%	400%	40%	200%
	5	6	7	8
Arc	20	20		
Len.	0.02	0.04		
Start	0	0		
End	120	120		
Hold	3	3		
Ramp	1000%	500%		

Notes
Bursty firing during test ∴ due to tracheal movements near the FCO.

REFERENCES

Aidley DJ (1978) *The Physiology of Excitable Cells* (Second Edition). Cambridge University Press, Cambridge, London, New York. 530 pp

Bacon JP, Altmann JS (1977) A silver intensification method for cobalt-filled neurones in wholemount preparations. *Brain Res* 138: 359-363

Ball EE (1981) Structure of the auditory system of the weta *Hemideina crassidens* (Blanchard, 1851) (Orthoptera, Ensifera, Gryllacridoidea, Stenopelmatidae). 2. Ultrastructure of the auditory sensilla. *Cell Tissue Res* 217: 345-359

Bässler U (1965) Proprioreceptoren am Subcoxal- und Femur-Tibia Gelenk der Stabheuschrecke *Carausius morosus* und ihre Rolle bei der Wahrnehmung der Schwerkraftrichtung. *Kybernetik* 2: 168-193

Bässler U (1976) Reversal of a reflex to a single motoneurone in the stick insect *Carausius morosus*. *Biol Cybern* 24: 47-49

Bässler U (1977) Sense organs in the femur of the stick insect and their relevance to the control of position of the femur-tibia joint. *J Comp Physiol* 121: 99-113

Bässler U (1979) Interactions of central and peripheral mechanisms during walking in first instar stick insects, *Extatosoma tiaratum*. *Physiol Entomol* 4: 193-199

Bässler U (1983) *Neural Basis of Elementary Behavior in Stick Insects. Studies of Brain Function*, Vol 10. Springer-Verlag, Berlin, 169 pp

Bässler U (1988) Functional principles of pattern generation for walking movements of stick insect forelegs: The role of the femoral chordotonal organ afferences. *J Exp Biol* 136: 125-147

Bässler U, Hofmann T, Schuch U (1986) Assisting components within a resistance reflex of the stick insect, *Cuniculina impigra*. *Physiol Entomol* 11: 359-366

Bate CM (1978) Development of sensory systems in arthropods. In: Jacobson M (Ed) *Handbook of Sensory Physiology*. Springer-Verlag, New York, pp 2-53

Bräunig P (1985) Strand receptors associated with the femoral chordotonal organs of locust legs. *J Exp Biol* 116: 331-341

Bräunig P, Hustert R, Pflüger HJ (1981) Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. I. Morphology, location and innervation of internal proprioceptors of pro- and metathorax and their central projections. *Cell Tissue Res* 216: 57-77

Bullock T, Horridge GA (1965) *Structure and Function of the Nervous Systems of Invertebrates.* WH Freeman & Co, San Fransisco & London. 1719 pp

Burns MD (1973) The control of walking in Orthoptera. I. Leg movements in normal walking. *J Exp Biol* 58: 45-58

Burns MD (1974) Structure and physiology of the locust femoral chordotonal organ. *J Insect Physiol* 20: 1319-1339

Burns MK, Usherwood PNR (1979) The control of walking in orthoptera. II. Motor neurone activity in normal free walking animals. *J Exp Biol* 79: 69-98

Burrows M (1980) The control of sets of motorneurones by local interneurones in the locust. *J Physiol Lond* 298: 213-233

Burrows M (1987) Parallel processing of proprioceptive signals by spiking local interneurons and motor neurons in the locust. *J Neurosci* 7(4): 1064-1080

Burrows M (1988) Responses of spiking local interneurones in the locust to proprioceptive signals from the femoral chordotonal organ. *J Comp Physiol* A 164: 207-217

Burrows M (1989) Processing of mechanosensory signals in local reflex pathways of the locust. *J Exp Biol* 146: 209-227

Burrows M, Hoyle G (1973) Neural mechanisms underlying behaviour in the locust *Schistocerca gregaria*. III Topography of limb motoneurons in the metathoracic ganglion. *J Neurobiol* 4: 167-186

Burrows M, Laurent GJ, Field LH (1988) Proprioceptive inputs to nonspiking local interneurons contribute to local reflexes of a locust hindleg. *J Neurosci* 8: 3085-3093

Büschges A (1989) Processing of sensory input from the femoral chordotonal organ by spiking interneurones of stick insects. *J Exp Biol* 144: 81-111

Campbell JI (1961) The anatomy of the mesothorax of *Locust migratoria migratoriodes* R. & F. *Proc Zool Soc Lond* 137: 403-432.

Cohen MJ (1960) The response patterns of single receptors in the crustacean statocyst. *Proc Roy Soc Lond* B152: 30-49

Cohen MJ, (1963) The crustacean myochordotonal organ as a proprioceptive system. *Comp Biochem Physiol* 8: 223-243

Cohen MJ, Hagiwara S, Zotterman Y (1955) The response spectrum of taste fibres in the cat: a single fibre analysis. *Acta Physiologica Scand* 33: 316-332

Coillot JP, Boistel J (1968) Localisation et déscription des récepteurs à l'étirement au niveau de l'articulation tibio-femorale de la patte sauteuse du criquet, *Schistocerca gregaria*. *J Ins Physiol* 14: 1661-1667

Coillot JP, Boistel J (1969) Étude de l'activité électrique propagée des récepteurs à l'étirement de la patte métathoracique du criquet, *Schistocerca gregaria*. *J Ins Physiol* 15: 1449-1470

Debaissieux P (1938) Organes scolopidiaux des pattes d'insectes. II. *La cellule* 47: 79-202

Debrodt B, Bässler U (1990) Responses of flexor motor neurones to stimulation of the femoral chordotonal organ of the phasmid *Extatosoma tiaratum*. *Zool Jb Physiol* 94: 101-119

Eggers F (1928) Die stiftfuehrenden Sinnesorgane. Morphologie und Physiologie der chordotonalen und tympanalen Sinnesapparate der Insecten. *Zoologische Bausteine* 2: 1-354

Evoy WH, Cohen MJ (1969) Sensory and motor interaction in the locomotor reflexes of crabs. *J Exp Biol* 51: 151-169

Field LH, Burrows M (1982) Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. *J Exp Biol* 101: 265-285

Field LH, Pflüger HJ (1989) The femoral chordotonal organ: A bifunctional Orthopteran (*Locusta migratoria*) sense organ ? *Comp Biochem Physiol* 93A: 729-743

Field LH, Rind FC (1981) A single insect chordotonal organ mediates inter- and intra-segmental leg reflexes. *Comp Biochem Physiol* 68A: 99-102

Forman RR, Zill SN (1984) Leg position learning by an insect: II motor strategies underlying learned leg extension. *J Neurobiol.* 15: 221-237

Füller H, Ernst A (1973) Die Ultrastruktor der femoralen Chordotonalorgane von *Carausius morosus* Br. *Zool Jb Anat Bd* 91: 574-601

Grosch A, Callender F, Petersen M, Cokl A, Kalmring K (1985) Vibration receptors of larvae and imagines in locusts: Location on the legs, central projections and physiology. In: Kalmring K, Elsner N (Eds) *Acoustical & Vibrational Communication in Insects*. Paul Parey, Hamburg, pp 151-161

Hartman HB, Boettiger G (1967) The functional organisation of the propus-dactylus organ in *Cancer irroratus* Say. *Comp Biochem Physiol* 22: 651-663

Heitler WJ, Burrows M (1977) The locust jump: I. The motor programme. *J Exp Biol* 66: 203-219

Hofmann T, Koch UT (1985) Acceleration receptors in the femoral chordotonal organ in the stick insect *Cuniculina impigra*. *J Exp Biol* 114: 225-237

Hofmann T, Koch UT, Bässler U (1985) Physiology of the femoral chordotonal organ in the stick insect *Cuniculina impigra*. *J Exp Biol* 114: 207-223

Howse PE (1968) The fine structure and functional organization of chordotonal organs. *Symp Zool Soc Lond* 23: 167-198

Hoyle G (1964) Exploration of neuronal mechanisms underlying behavior in

insects. In: Reiss RF (Ed) *Neural Theory and Modeling (Proceedings of the 1962 Ojai symposium)*. Stanford University Press, Stanford. pp 346-376

Hubbard SJ (1959) Femoral mechanoreceptors in the locust. *J Physiol Lond* 147: 8-10

Hustert R (1978) Segmental and interganglionic projections from primary fibres of insect mechanoreceptors. *Cell Tissue Res* 194: 337-351

Hustert R (1982) The propriceptive function of a complex chordotonal organ associated with the mesothoracic coxa in locusts. *J Comp Physiol* 147: 389-399

Hustert R, Pflüger HJ, Bräunig P (1981) Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. III. The external mechanoreceptors: The campaniform sensilla. *Cell Tissue Res* 216: 97-111

Johnson SE, Murphey RK (1985) The afferent projection of mesothoracic bristle hairs in the cricket, *Acheta domesticus*. *J Comp Physiol* A 156: 369-379

Kalmring K, Lewis B, Eichendorf A (1978) The physiological characteristics of the primary sensory neurons of the complex tibial organ of *Decticus verrucivorus* L. (Orthoptera, Tettigonioidae). *J Comp Physiol* 127: 109-121

Kühne R (1982) Neurophysiology of the vibration sense in locusts and bushcrickets: response characteristics of single receptor units. *J Insect Physiol* 28: 155-163

Laurent G (1986) Thoracic intersegmental interneurones in the locust with mechanoreceptive inputs from a leg. *J Comp Physiol* A 159: 171-186

Laurent G (1987) Parallel effects of joint receptors on motor neurones and intersegmental interneurones in the locust. *J Comp Physiol* A 160: 341-353

Laurent G (1988) Local circuits underlying excitation and inhibition of intersegmental interneurones in the locust. *J Comp Physiol* A 162: 145-157

Laurent G, Burrows M (1988) A population of ascending intersegmental interneurones in the locust with mechanosensory inputs from a hind leg. *J Comp Neurol* 275: 1-12

Lowenstein O, Roberts TDM (1950) The equilibrium function of the otolith organs of the thornback ray (*Raja clavata*). *J Physiol* 110: 392-415

Lutz EM, Tyrer NM (1988) Immunohistochemical localization of serotonin and choline acetyltransferase in sensory neurones of the locust. *J Comp Neurol* 267: 335-342

Macmillan DL, Kien J (1983) Intra- and intersegmental pathways active during walking in the locust. *Proc Roy Soc Lond* B 218: 287-308

Matheson T (1990) Responses and locations of neurones in the locust metathoracic femoral chordotonal organ. *J Comp Physiol* A166: 915-927

Mill PJ (1976) Chordotonal organs of crustacean appendages. In: Mill PJ (Ed) *Structure and Function of Proprioceptors in the Invertebrates*. Chapman & Hall, London. pp 243-297

Mill PJ (Ed) *Structure and Function of Proprioceptors in the Invertebrates*. Chapman & Hall, London. 686 pp

Mill PJ, Lowe DA (1972) An analysis of the types of sensory unit present in the PD proprioceptor of decapod crustaceans. *J Exp Biol* 56: 509-525

Mill PJ, Lowe DA (1973) The fine structure of the PD proprioceptor of *Cancer pagurus*. II. The receptor strand and the movement sensitive cells. *Proc Roy Soc Lond* B 184: 179-197

Moran DT, Rowley III JC, Varela FG (1975) Ultrastructure of the grasshopper proximal femoral chordotonal organ. *Cell Tissue Res* 161: 445-457

Moran DT, Varela FJ, Rowley JC (1977) Evidence for active role of cilia in sensory transduction. *Proc Nat Acad Sci* 74: 793-797

Moulins M (1976) Ultrastructure of chordotonal organs. In: Mill PJ (Ed) *Structure and Function of Proprioceptors in the Invertebrates*. Chapman & Hall, London. pp 387-426

Murphey RK, Jacklet A, Schuster L (1980) A topographic map of sensory cell terminal arborizations in the cricket CNS: correlation with birthday and position in a sensory array. *J Comp Neurol* 191: 53-64

Newland P (1989) Morphology and physiology of ascending interneurones in the metathoracic ganglion of the locust. In Erber J, Menzel R, Pflüger H-J, Todt D (Eds) *Neural Mechanisms of Behavior. Proceedings of the 2nd International Congress of Neuroethology.* Georg Thieme Verlag. Stuttgart, New York

Oldfield BP (1983) Central projections of primary auditory fibres in tettigoniidae (Orthoptera: Ensifera). *J Comp Physiol* 151: 389-395

Orchard I (1975) The structure and properties of an abdominal chordotonal organ in *Carausius morosus* and *Blaberus discoidalis*. *J Ins Physiol* 21: 1491-1499

Pflüger HJ, Burrows M (1987) A strand receptor with a central cell body synapses upon spiking local interneurones in the locust. *J Comp Physiol* 160: 295-304

Pflüger HJ, Bräunig P, Hustert R (1981) Distribution and specific central projections

of mechanoreceptors in the thorax and proximal leg joints of locusts. II. The external mechanoreceptors: Hair plates and tactile hairs. *Cell Tissue Res* 216: 79-96

Pflüger HJ, Bräunig P, Hustert R (1988) The organization of mechanosensory neuropiles in locust thoracic ganglia. *Phil Trans Roy Soc Lond* B 321: 1-26

Pflüger, HJ, Bräunig P, Hustert R (1988) The organization of mechanosensory neuropiles in locust thoracic ganglia. *Phil Trans Roy Soc Lond* B 321: 1-26

Rheinlaender J (1975) Transmission of acoustic information at three neuronal levels in the auditory system of *Decticus verrucivorus* (Tettigoniidae, Orthoptera). *J Comp Physiol* 97: 1-53

Römer H (1985) Anatomical representation of frequency and intensity in the auditory system of Orthoptera. In: Kalmring K, Elsner N (Eds) *Acoustical & Vibrational Communication in Insects*. Paul Parey, Hamburg, pp 25-32

Römer H, Marquart V, Hardt M (1988) Organization of a sensory neuropile in the auditory pathway of two groups of orthoptera. *J Comp Neurol* 275: 201-215

Runion HI, Usherwood PNR (1966) Responsiveness of a mechanoreceptor (chordotonal organ) in the locust leg to static displacement and velocity of stretch. *J Physiol Lond* 187:40P-41P

SAS User's Guide: Statistics. Version 5 Edition (1985) SAS Institute Inc. Cary, NC, USA

Shimozawa T, Kanou M (1984) The aerodynamics and sensory physiology of range fractionation in the cercal filiform sensilla of the cricket *Gryllus bimaculatus*. *J Comp Physiol* A155: 495-505

Shimozawa T, Kanou M (1984) Varieties of filiform hairs: range fractionation by sensory afferents and cercal interneurons of a cricket. *J Comp Physiol* A155: 485-493

Siegler MVS (1984) Local interneurones and local interactions in arthropods. *J Exp Biol* 112: 253-281

Slifer EH (1935) Morphology and development of the femoral chordotonal organs of *Melanoplus differentialis* (Orthoptera, Acrididae). *J Morph* 58: 615-637

Slifer EH, Sekhon SS (1975) The femoral chordotonal organs of a grasshopper, Orthoptera, Acrididae. *J Neurocytol* 4: 419-438

Taylor RC (1975a) Physical and physiological properties of the crayfish antennal flagellum. *J Neurobiol* 6: 501-519

Taylor RC (1975b) Integration in the crayfish antennal neuropile: Topographic

representation and multiple channel coding ofthe mechanorecepto submodalities. *J Neurobiol* 6: 475-499

Theophilidis G (1986a) The femoral chordotonal organs of *Decticus albifrons* (Orthoptera: Tettigoniidae) - I. Structure. *Comp Biochem Physiol* 84A: 529-536

Theophilidis G (1986b) The femoral chordotonal organs of *Decticus albifrons* (Orthoptera: Tettigoniidae) - II. Function. *Comp Biochem Physiol* 84A: 537-543

Usherwood PNR, Runion HI, Campbell JI (1968) Structure and physiology of a chordotonal organ in the locust leg. *J Exp Biol* 116: 435-461

Weiland G, Koch UT (1987) Sensory feedback during active movements of stick insects. *J Exp Biol* 133: 137-156

Wiersma CAG, Boettiger EG (1959) Unidirectional movement fibres from a proprioceptive organ of the crab, *Carcinus maenas*. *J Exp Biol* 36: 102-112

Wilson JA (1981) Unique, identifiable local non spiking interneurones in the locust mesothoracic ganglion. *J Neuriobiol* 12: 353-366

Wolfrum U (1990) Actin filaments: the main components of the scolopale in insect sensilla. *Cell Tiss Res* 261: 85-96

Young D (1970) The structure and function of a connective chordotonal organ in the cockroach leg. *Phil Trans Roy Soc Lond* B 256: 401-426

Young D (1975) Chordotonal organs associated with sound producing apparatus of cicadas (Insecta: Homoptera). *Z Morph Tiere* 81: 111-135

Zill SN (1985a) Plasticity and proprioception in insects. I. Responses and cellular properties of individual receptors of the locust metathoracic femoral chordotonal organ. *J Exp Biol* 116: 435-461

Zill SN (1985b) Plasticity and proprioception in insects. II. Modes of reflex action of the locust metathoracic femoral chordotonal organ. *J Exp Biol* 116: 463-480

Zill SN (1987) Selective mechanical stimulation of an identified proprioreceptor in freely moving locusts: role of resistance reflexes in active posture. *Brain Res* 417(1): 195-198

Zill SN, Jepson-Innes K (1988) Evolutionary adaptation of a reflex system: sensory hysteresis counters muscle 'catch' tension. *J Comp Physiol* A164: 43-48

Zill SN, Moran DT (1981) The exoskeleton and insect proprioception. I. The responses of tibial campaniform sensilla to external and muscle-generated forces in the american cockroach *Periplaneta americana*. *J Exp Biol* 91: 1-24